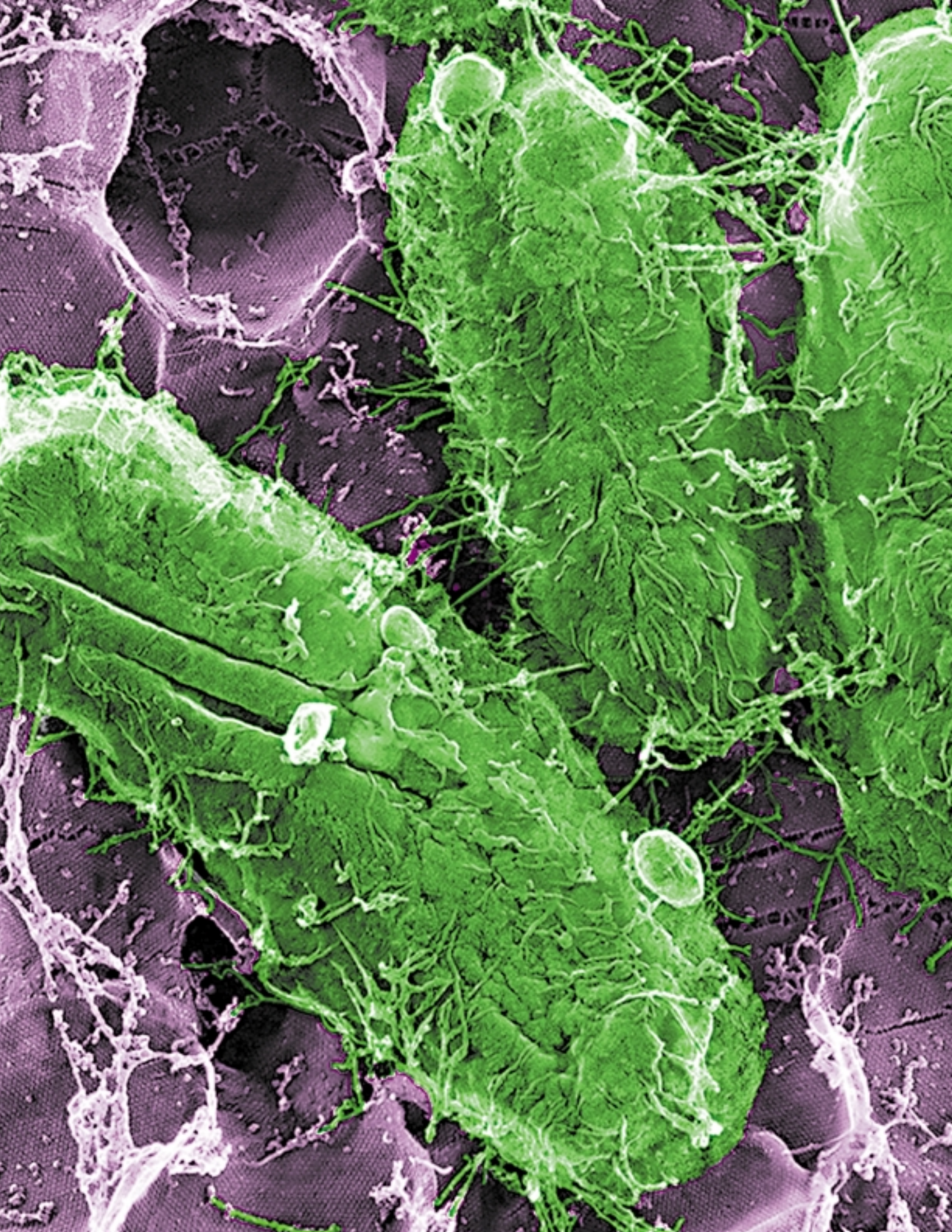


Kidney, Urologic and Blood Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health care problems in the United States. They cause suffering and disability for millions of Americans, including children and young adults. Many diseases in this category are more common in African American, Native American or Hispanic populations, and the reasons for this are not known. The NIDDK is dedicated to research aimed at understanding, treating and preventing these diseases.

Gene Discoveries Spur Advances in Polycystic Kidney Disease

Polycystic kidney disease (PKD) is a genetic disease characterized by massive enlargement of the kidneys, which is caused by the growth of multiple fluid-filled cysts. It is estimated that PKD affects as many as 500,000 to 600,000 people in the United States, and is the fourth leading cause of kidney failure.

A landmark advance in PKD research occurred when scientists discovered the genes, known as *PKD1* and *PKD2*, which, when mutated, are responsible for the development of the most prevalent form of PKD. Since discovery of these causative genes, investigators using three mouse models of the disease have made several advances in understanding PKD. They discovered a family of proteins produced by the PKD genes, called polycystins. In the most common form of PKD, the severity of the mutation was directly related to whether the animals died before birth, or had decreased life spans. They concluded that the presence of polycystin-2 is essential for normal development of parts of the kidney, heart, and pancreas. A second research team examined kidney cysts from two patients and discovered that 71 percent of the cysts had mutations in the *PKD2* gene, while a subset of cysts lacked that mutation but had mutations in the *PKD1* gene. The findings suggest that *PKD1* mutations may be modifiers of disease severity, and that independent disturbances in the production of the polycystin proteins by the PKD genes may be sufficiently disruptive to cause cyst formation. In another series of experiments using a mouse model, researchers built on insights in cell signaling mechanisms to show that a growth factor inhibitor, EKI-785, reduced cysts, improved kidney function, decreased liver abnormalities, and increased life span. The drug acts on an enzyme critical for growth factor signaling. The disease returned when the mice were no longer administered this drug.

The discovery of the PKD genes has also opened new avenues of research. These include the determination of the proteins produced by the genes and how they function in the normal and disease states. Pursuit of these research avenues is helping to advance understanding of the molecular and cellular events in PKD, so that safe and effective therapies can be developed. In addition, further research on growth factor signaling pathways could form the basis for starting clinical trials in patients with the goal of developing an effective treatment for PKD.

NIDDK support for PKD research is being strengthened by new work on mouse models and on basic cell biology, in order to understand the cause of disease and to facilitate testing new treatment interventions. Four centers for research on PKD have recently been established. Under a new initiative, the NIDDK is providing support and coordination of research efforts for the

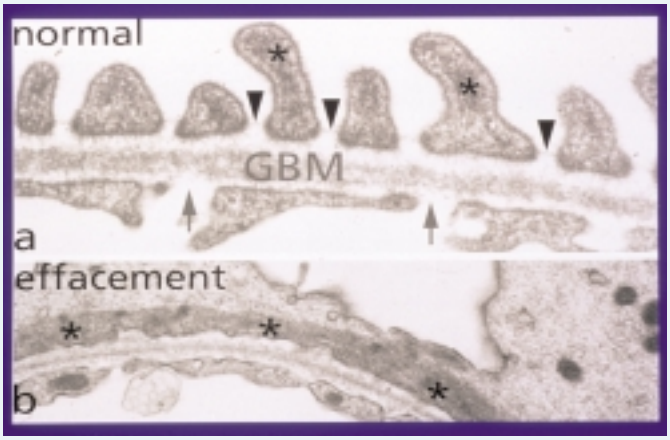
(Continued on page 82.)

Urinary tract and bladder infections are caused by bacteria. In this photo, a harmful strain of *E. coli* (green) attaches itself to the lining of the bladder (purple) though the use of pili, thread-like extensions that help the bacteria hold onto the cells of the bladder wall. NIDDK-supported researchers are working to identify the precise mechanism by which the bacteria infect cells and to develop new ways to prevent infection. Vaccination against pilus proteins may enable humans to fight off bladder infections. Photo: Mulvey MA, Schilling JD, Martinez JJ, and Hultgren SJ. From the cover: Bad bugs and beleaguered bladders: Interplay between uropathogenic *Escherichia coli* and innate host defenses. *PNAS* 97:8829-35, 2000.

Cells with Feet

Normal kidneys work very efficiently to cleanse the blood of waste products and retain normal blood constituents—water, salts, and blood proteins. The first step in this process is filtration of the blood plasma by the renal glomerulus, a complex structure consisting of a tiny ball of delicate capillaries surrounded by special cells called *podocytes*. The human kidney has a million of these tiny filters. Massive quantities of fluid are filtered from the blood by the glomeruli, about one hundred and eighty liters per day, but very little protein escapes. Blood proteins do not leak out because the filtration properties of the glomerulus result in retention of large molecules such as albumin and other proteins. The podocyte—the word means cell with feet—is a critical component of this filter. New evidence establishes that it plays the major role in synthesizing the scaffold that lets fluid through and holds back blood proteins; it is the key to maintaining the integrity of the filter.

Physicians have long been aware that leakage of protein into the urine is an important early sign of kidney disease, but the significance of even small amounts of protein in the urine as a risk factor for eventual kidney failure has only recently been appreciated. In the last few years, several large clinical trials have examined the progression of kidney dysfunction to kidney failure. It has been found that patients with small amounts of protein in the urine are much more likely to progress to kidney failure than other patients who have equal degrees of kidney dysfunction but who do not have protein in the urine. In patients with diabetes, the earliest warning of the presence of kidney involvement is the appearance of small amounts of albumin in the urine. Recent studies show that reduction in the amount of albumin in the urine is associated with stabilization of disease, and increases in albumin and other urine proteins are linked to development of kidney failure.



Podocytes—"cells with feet"—play a critical role in the filtration of blood and the removal of waste products for excretion. As shown in the top half of the above photo (a), normal podocytes atop the kidney's glomerular basement membrane (GMB) form an important barrier that filters blood and other fluid, but impedes protein from leaking into the urine. The bottom half of this photo (b) shows the effacement of podocytes in nephrotic syndrome. When this happens, the filtration barrier does not function properly. As a result, protein leaks into the urine and renal failure can develop. Photo: Somlo S, and Mundel P. Getting a foothold in nephrotic syndrome. *Nat Genet* (24):333-335, 2000

Another important condition characterized by protein in the urine is nephrotic syndrome. The end result of a variety of diseases, this condition is characterized by massive loss of blood proteins into the urine. This loss causes a variety of disturbances in body function, including retention of salt and water, and the development of high blood pressure and high cholesterol. One of the most common forms of nephrotic syndrome occurs in children. In some rare cases, it runs in families. Currently, treatment of nephrotic syndrome focuses on identifying the underlying cause, if possible, and on reducing high cholesterol, blood pressure, and protein in the urine through diet, medications, or both. One group of blood pressure medications, known as ACE inhibitors, which also protects the kidneys of diabetic patients, is sometimes helpful for reducing proteinuria in nephrotic syndrome. In many cases, however, the proteinuria is resistant to treatment. All of these observations underscore the importance of understanding the glomerular filter and learning what retains proteins in the glomerulus and what causes leakiness.

Over several decades, basic research on the kidney glomerulus has yielded a clear description of the filtration barrier. The filter is composed of three layers: the cells lining the capillaries (endothelial cells), the podocytes (the cells with feet) and the glomerular base-

ment membrane, a collagenous gel that separates the two cell layers. Stretching between the "feet" of the podocytes is the "slit diaphragm," the principal filtration barrier to large blood proteins.

The first genetic defect established to cause leaking of proteins into the urine was in the collagen molecules that make up the glomerular basement membrane. Defects in this form of collagen cause Alport's syndrome, a disorder in which protein leaks into the urine and may cause kidney failure. Kidney biopsies from patients with Alport's syndrome show very thin glomerular basement membranes. In most conditions in which there is protein in the urine, however, the glomerular basement membranes appear structurally normal.

Increasingly, research interest has focused on the podocyte and on understanding the special molecules that the podocyte uses to form the slit diaphragm and maintain the filtration barrier. In the past three years there has been a rapid series of important discoveries in understanding the molecular basis of podocyte function and malfunction. These insights have emerged primarily from the application of genetic approaches to either man or mouse, but they have been helped by decades of studies in rats and insights from the genome of the roundworm.

The first of these observations occurred in 1998, when scientists identified the gene mutated in a rare type of hereditary nephrotic syndrome found in Finnish families. The gene's protein product, called nephrin, is expressed only in the podocyte and is a major component of the slit diaphragm filtration barrier.

In 1999, a second group of investigators discovered, unexpectedly, that massive proteinuria and kidney failure developed in mice that were genetically altered to lack a protein named CD2 adaptor protein (CD2AP), a protein not previously known to be important in kidney function. In fact, prior to these studies, CD2AP was thought to function only as a T lymphocyte protein and part of the immune system. The researchers found that, in fact, CD2AP interacts with nephrin in the slit diaphragm, and suggested that CD2AP may serve to anchor nephrin to the podocyte.

Then, in March 2000, scientists reported the discovery—again using genetic approaches—of a third protein, named alpha-actinin-4, that is produced by a gene located close to the nephrin gene. Mutations in this gene are associated with focal sclerosis, a kidney disease that leads to nephrotic syndrome and kidney failure in adulthood. The investigators showed that mutant alpha-actinin-4 actually binds more strongly to a protein in the podocyte that helps maintain the shape of the cell's foot processes than does alpha-actinin-4 from unaffected individuals. The mutant alpha-actinin-4 also is produced at higher levels in podocytes. They speculate that altered alpha-actinin-4 may act in a dominant fashion to alter podocyte foot shape, thereby altering the structure of the slit diaphragm and glomerular function, resulting in slow development of kidney damage.

Closely following these observations was another report in April 2000, of a fourth gene which, when mutated, is associated with protein in the urine. The gene produces a membrane protein, named podocin, and again, it is exclusively expressed in the podocyte. It was found to be defective in thirteen families with a form of nephrotic syndrome that presents in infancy and leads rapidly to kidney failure. While the precise function of podocin is not yet known, it is very similar to a protein called MEC-2 found in the roundworm, *C. elegans*. MEC-2 functions to link stretch-sensitive ion channels on the cell surface with internal skeletal structures of the cell. Thus, these investigators suggested that podocin may be important for cell-surface interactions critical in maintaining the shape of podocyte foot processes. This research is an example of how detailed knowledge of a simpler, non-mammalian model system has helped to accelerate the pace of human disease research. In the case of *C. elegans*, investigators will be able to perform studies of the podocin-like protein to define its interactions with other proteins. This information will bear directly on the study of the filtration barrier in humans.

Studies of these four proteins—nephrin, CD2AP, alpha-actinin-4, and podocin—are revolutionizing the fundamental knowledge of the molecular mechanisms of glomerular filtration. Because abnormal function of the filtration barrier is a major complication in most clinically important kidney diseases, such as hypertensive nephropathy and diabetic kidney disease, further studies of these proteins hold great promise for suggesting new strategies for treatment and prevention.

(Continued from page 79.)
development and testing of improved state-of-the-art imaging methods for PKD. The objective is to test whether such techniques can provide accurate and reproducible markers of progression of renal disease in PKD, and especially of the proportion of the kidney occupied by cysts. The hope is that these techniques will facilitate testing of interventional strategies to slow or prevent disease progression in PKD.

Sweeney WE, Chen Y, Nakanishi K, Frost P, and Avner ED. Treatment of polycystic kidney disease with a novel tyrosine kinase inhibitor. *Kidney Int* 57(1):33-40, 2000.

Watnick T, He N, Wang K, Liang Y, Parfrey P, Hefferton D, St. George-Hyslop P, Germino G, and Pei Y. Mutations of *PKD1* in ADPKD2 cysts suggest a pathogenic effect of trans-heterozygous mutations. *Nat Genet* 25(2):143-144, 2000.

Wu G, Markowitz GS, Li L, D'Agati VD, Factor SM, Geng L, Tibara S, Tuchman J, Cai Y, Park JH, van Adelsberg J, Hou H Jr, Kucherlapati R, Edelmann W, and Somlo S. Cardiac defects and renal failure in mice with targeted mutations in *PKD2*. *Nat Genet* 24(1):75-78, 2000.

Lifesaving Technologies: Kidney Dialysis and Transplantation

The kidneys are critically important organs in the body's waste filtration system. If the kidneys fail, human life cannot be sustained without either kidney dialysis—in which mechanical equipment is used to filter the blood—or kidney transplantation, in which a donated kidney replaces the one destroyed by disease. Kidney disease, diabetes, and high blood pressure are linked in a dangerous triangle. Diabetes is the primary cause of kidney failure, and kidney failure results in high blood pressure and other cardiovascular complications. High blood pressure resulting from other diseases, including diabetes, can also cause kidney failure. The NIDDK funds an extensive body of research aimed at understanding the underlying mechanisms of both diabetes and kidney disease, with the goal of developing effective treatments and possible methods of prevention.

One recent study focused on interstitial nephritis, a medical term for a type of kidney inflammation. Symptoms include an increase or decrease in urination, fever, fluid retention, change in mental state (confusion or drowsiness), nausea and vomiting. Usually, nephritis lasts for only a short time (acute) and the cause is readily identifiable. The most common cause is a reaction to medication, including over-the-counter anti-inflammatory drugs and antibiotics. Patients suffering from diabetes, high blood pressure, or certain kidney diseases may also have interstitial nephritis. Sometimes, however, kidney inflammation lasts for a long time (chronic), and the cause is not easy to identify. Diseases of unknown origin are termed idiopathic, meaning, "cause unknown." Scientists recently reported that the kidneys of patients suffering from idiopathic chronic interstitial nephritis were infected with the Epstein-Barr virus (EBV), which causes inflammation and may lead to chronic disease due to tissue damage. Thus, EBV infection may be one possible cause of idiopathic chronic interstitial nephritis when patients are not suffering from other diseases or taking medications thought to cause kidney inflammation.

Kidney disease takes many forms, some of which progress to irreversible kidney failure, known as end stage renal disease (ESRD). While kidney dialysis will save the life of a patient with ESRD, it is a difficult and time-consuming treatment to administer. Moreover, the life-expectancy of dialysis patients, while greatly improved, is still relatively short. The alternative to long-term dialysis for ESRD is kidney transplantation. However, not all patients are good candidates for transplantation, and the risks and benefits of transplantation *versus* dialysis must be considered. For example: Who will benefit the most from a kidney transplant? What happens to children who undergo long-term dialysis? What factor is more important: how closely the donor kidney matches the recipient's tissue type, or how soon the kidney is transplanted? Ongoing NIDDK-supported studies are attempting to address these problems and to broaden understanding of the effects of dialysis and transplantation on patients so that this knowledge can inform medical practice.

For example, a group of NIDDK-supported investigators performed a study of dialysis patients under age 30 to determine whether they have the same type of increase in calcium deposits within the coronary arteries, the blood vessels surrounding the heart, that has been observed in adults on long-term dialysis. The investigators found that young patients did indeed have evidence of increased calcium deposits in their coronary arteries, and the severity of the deposits was directly linked to duration of dialysis. The young patients had more calcium deposits than either age-matched or older patients who had normal kidney function. Moreover, when tested again the follow-

ing year, patients with high initial scores indicative of calcium deposits were found to have nearly twice their previous scores. These data suggest that the cardiovascular systems of young adults undergoing dialysis are subject to ongoing detrimental effects. In clinical terms, this means that patients whose kidneys fail early in life, and thus begin dialysis early, may profit from therapies aimed at decreasing calcification of coronary arteries.

Another recent study relates to an important goal in kidney transplantation–efforts to maximize patient health and survival, while minimizing costs. Researchers examined transplant patients' Medicare costs as a result of the degree of donor-to-patient tissue-type matching and the length of time the kidney is held in cold storage prior to transplantation. The study concluded that better-matched kidney transplants result in better patient health and lower costs, whereas increased storage time results in poorer patient health and higher costs. In related research, NIDDK-supported investigators compared death rates of patients on dialysis to those of patients waiting for or receiving kidney transplants. The hypothesis was that patients selected to be on a transplant waiting list are healthier initially and thus less likely to die than those not placed on a waiting list. After analyzing patient information from the NIDDK's U.S. Renal Data System (USRDS), researchers confirmed that dialysis patients added to a waiting list for kidney transplants are indeed less likely to die than dialysis patients not selected to wait for transplants. Moreover, long-term survival of those on the waiting list who receive transplants is greater than the survival of those who remain on the waiting list. The researchers also determined that, although diabetes is the predominant cause of kidney failure, diabetic patients were the most likely to have a projected increase in life span following transplantation. This study provides important data to doctors regarding significant aspects of kidney transplantation and may thus help inform medical choices and decisions, for the ultimate benefit of patients.

The NIDDK continues to fund projects designed to improve patients' quality of life and assist doctors in their treatment decisions. Ongoing projects include the U.S. Renal Data System (mentioned previously), and a hemodialysis clinical trial designed to evaluate the effects of long-term dialysis on patient health and survival. The USRDS is a database containing information about how many people have kidney disease, how they are being treated, and the outcomes of treatment. USRDS data are publicly available on the NIDDK World Wide Web site *(http://www.niddk.nih.gov)*. Future studies will attempt to improve doctors' ability to access the blood vessels of dialysis patients and to understand risk factors for developing ESRD and ESRD-related cardiovascular diseases. Because of the urgent need to halt the rising tide of renal failure, the NIDDK is launching a National Kidney Disease Education Program.

Becker JL, Miller F, Nuovo GJ, Josepovitz C, Schubach WH, and Nord EP. Epstein-Barr virus infection of renal proximal tubule cells: possible role in chronic interstitial nephritis. *J Clin Invest* 104(12):1673-1681, 1999.

Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D, Wang Y, Chung J, Emerick A, Greaser L, Elashoff RM, and Salusky IB. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *New Engl J Med* 342(20):1478-1483, 2000.

Schnitzler MA, Hollenbeak CS, Cohen DS, Woodward RS, Lowell JA, Singer GG, Tesi RJ, Howard TK, Mohanakumar T, and Brennan DC. The economic implications of HLA matching in cadaveric renal transplantation. *New Engl J Med* 341(19):1440-1446, 1999.

Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, Held PJ, and Port FK. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *New Engl J Med* 341(23):1725-1730, 1999.

Photo: Mr. Richard Nowitz.

PATIENT PROFILE

Jill McMaster – Polycystic Kidney Disease

Jill McMaster's mother died of polycystic kidney disease (PKD), a dominantly inherited disease, when Jill was 16. Jill knew there was a 50 percent chance she might have PKD as well. But because her older brother and sister and both of her first cousins had all been diagnosed with the disease, Jill thought her chances of beating the odds were pretty good. Unfortunately, that was not to be the case. When Jill was 24, and asymptomatic at the time, an ultrasound examination, administered during a research study of families genetically predisposed to PKD, revealed cysts on her kidneys.

"Prior to my diagnosis, and knowing the course this disease takes, my major concern was: who will I eventually donate one of my kidneys to, my brother or sister," says Jill. Now 49, Jill is a senior researcher with the Federal government, and received her own kidney transplant two years ago. She is also a Polycystic Kidney Research Foundation board member, and her life's mission is to advocate to find a cure for this deadly disease.

PKD is the fourth leading cause of kidney failure in the U.S. Complications of the disease can include life-ending brain aneurysms, mitral valve prolapse, enlarged heart, and polycystic ovaries or polycystic testes. Patients commonly have high blood pressure, infections, and chronic flank or back pain. There is no known treatment to prevent patients with PKD from developing kidney failure.

Fortunately for Jill and the other hundreds of thousands of people in the U.S. affected with PKD, research on this disease and on the treatment of kidney failure is progressing rapidly—with support from the NIDDK.

- The recent breakthrough discovery of the genes responsible for PKD has opened new avenues of research, including the identification of proteins produced by the genes and how they function in normal and diseased states.

Jill McMaster was diagnosed with polycystic kidney disease at age 24. She underwent a kidney transplant two years ago and now, at age 49, works to help find a cure for this disease. Jill's life improved dramatically following her transplant. "I could once again feel a belly laugh, and it felt good."

- New animal models are helping to unravel the cellular aspects of PKD, how the disease-causing genes function, and whether potential therapies show promise of effectiveness.

- Dietary and nutritional interventions are being studied as ways to slow the growth of cysts.

- Systematic studies are under way to provide accurate assessment of the clinical progression of the disease, in order to plan and test clinical interventions for PKD.

- Novel approaches are under investigation to prevent the rejection of kidneys transplanted into patients with end-stage renal disease, including PKD patients whose kidneys have failed.

What We Know—and Don't Know—About PKD

No one knows for sure what causes PKD. What is known is that the cysts grow out of the tubules, the tiny conduits for processing and delivering fluid inside the kidneys. The cysts eventually fill with fluid, and continue to enlarge. While generally retaining their shape, the kidneys enlarge along with the cysts—which can number in the thousands. The cysts squeeze out the

nephrons, hindering their function, and impairing kidney function overall.

A common misconception of genetic diseases, including PKD, is that a child's risk of inheriting a disease from parents is lower if the child's siblings already have the disease. In fact, the risk is still 50 percent regardless of the status of family members. Mutations in two genes, *PKD1* and *PKD2*, have been identified as the cause of the two most common forms of PKD. These genes produce proteins, termed polycystins, whose normal function is important for kidney development, and whose abnormal (mutated) function leads to cyst formation. Research indicates that one form of polycystin is important for the regulation of calcium transport in the cell. Scientists think that abnormal polycystin may cause alteration in calcium transport that can lead to abnormal fluid secretion, resulting in cellular growth, or the development of cysts. This type of information is critical to developing new treatments for the disease, including gene therapy.

How do these fluid-saturated cysts affect people with PKD? Consider this. Normal kidneys weigh approximately eight ounces. At the time of her transplant, Jill's fluid-filled cysts had enlarged her kidneys to approximately 14 pounds. They were so large, she says, that they placed enormous pressure on her stomach, forcing her to eat several small meals a day. Her diet included a great deal of water, very little salt, and very few caffeinated beverages.

Formerly a slim, athletic woman, Jill, at a certain point in the progress of the disease, had to shift to low-impact sports such as swimming, and because of her enlarged kidneys, couldn't find clothes that fit. Her self- image suffered. "For many years prior to my transplant I looked pregnant," she says. "I often wore maternity clothes for comfort." Despite all that, she feels fortunate.

"I'm one of the lucky ones," she explains. "My pain was limited, and I continued to work full time up until I got the call for my transplant." Some people with PKD are forced to stop working, and as their kidneys fail to properly clean their blood, the disease impacts the brain, which may bring about severe depression.

Jill was also fortunate because a kidney was found for transplantation before she would have had to go on dialysis. Those patients who do develop kidney failure and do not receive a transplant, must undergo either hemodialysis, which takes over the function of diseased kidneys by cleaning and filtering the blood, or peritoneal dialysis, which removes excess fluid, electrolytes, and wastes from the body, using the lining of the abdominal cavity. Though laborious and time-consuming for the patient, dialysis is a life-saving therapy.

What the Future Holds

No one knows for sure when a treatment or cure for PKD will be found. But new research discoveries are continually being made, raising the hopes of Jill and others with the disease. For example, the antibiotic

PKD FACTS

- **PKD affects as many as 500,00 to 600,000 people in the U.S., and is the fourth leading cause of kidney failure.**
- **Autosomal Dominant Polycystic Kidney Disease (ADPKD), the most common of all life-threatening genetic diseases, equally affects people regardless of sex, race, age, or ethnic origin.**
- **There is no known treatment or cure for PKD directed at the basic mechanism of the disease.**
- **PKD can be passed from one generation to the next by an affected parent, and it does not "skip" a generation as do some genetic diseases.**
- **Medicare/Medicaid costs for kidney dialysis, transplantation, and related therapies for kidney failure caused by PKD approach $2 billion annually.**

PATIENT PROFILE

ciprofloxicin is now being used to treat infections that occur inside the cysts. Ciprofloxicin reduces infection that causes bleeding, leading to organ enlargement and pain. Of significance, too, was the recent discovery that an existing cancer drug used on laboratory animals with one form of PKD virtually stopped disease progression.

Jill knows her kidney transplant won't last forever. "I'm hoping to get 20 to 25 years out of it," she says. She's looking to the NIDDK and to organizations such as the Polycystic Kidney Research Foundation to advance a treatment. Meanwhile, she's going to continue to speak out and work on behalf of a cure for PKD, as well as appreciate the little things in life. "After my transplant, when my enlarged PKD kidneys were removed," she says, "I again had room to breathe naturally. I could once again feel a belly laugh, and it felt good."

Easing the Pain of Kidney Stones

Kidney stones can cause excruciating pain and many patients suffer from recurrent episodes. Each year more than a million people are diagnosed with kidney stones, making them one of the most prevalent problems of the urinary system. Caucasians are more likely to develop stones than African Americans, and men develop them more often than women. Stones can cause such severe pain because they frequently block the flow of urine or become lodged in the kidneys or narrow tubes of the urinary system. Symptoms of kidney stones include shooting pain in the back, profuse sweating, blood in the urine, and the frequent urge to urinate. Women suffering from kidney stones describe the pain as being "worse than childbirth."

What are kidney stones and what causes them? Kidney stones are solid masses that form out of substances excreted in urine. The exact cause of most kidney stones is unknown. Under normal conditions, chemicals within urine prevent stone formation, and wastes are excreted without being noticed. In some people, however, the stone-preventing chemicals fail to do their job. When this happens, crystals form and aggregate into stones. Sometimes this process may follow a recent kidney infection, but most often it occurs when the kidneys excrete too much of certain minerals, such as calcium. Less common types of stones are formed from uric acid or cystine. Because kidney stones run in families, a genetic defect may make some people more likely to develop stones than others. In addition, kidney stone formation can accompany certain diseases, such as urinary tract infections, cystic kidney disease, and hyperparathyroidism.

Excessive levels of calcium, present in a disease called hypercalciuria, can also cause kidney stone formation. In afflicted patients, the large intestine absorbs too much calcium. When the excess calcium is excreted in urine, kidney stones develop. This disease also runs in families, suggesting that it may be inherited via a genetic defect, although thus far the identity of the gene remains a mystery. Recently, a new clue emerged when a group of NIDDK-supported scientists examined three severely affected families. They identified in all family members with kidney stones a chromosomal region harboring a gene likely to be responsible for development of kidney stones in those affected by the disease. However, some family members with the presumed abnormal gene did not develop kidney stones. This finding suggests that, in addition to genetic inheritance, environmental factors and lifestyle probably also play a role in the development of kidney stones. The NIDDK supports work directed at figuring out the roles of both genetic inheritance and environment in the development of kidney stones. In pursuit of this goal, the NIDDK is bolstering efforts to encourage research into the genetic inheritance of kidney stone diseases.

Reed BY, Heller HJ, Gitomer WL, and Pak CY. Mapping a gene defect in absorptive hypercalciuria to chromosome 1q23.3-q24. *J Clin Endocrinol Metab* 84(11):3907-3913, 1999.

PATIENT PROFILE

Kyle Rosen – Pediatric Kidney Disease

At six weeks old, Kyle Rosen developed a 101.5-degree fever. Routine medical tests, including blood work, a urine sample, and sonogram indicated that both of Kyle's kidneys were swollen and that the infant also had a urinary tract infection, which required him to be hospitalized. Kyle's parents, Brett Rosen and Debra Wattenberg, were told by attending physicians that this wasn't too uncommon in infants. They recommended that Kyle take an antibiotic to prevent further kidney infection and then be examined again in six months. Debra, herself a physician, wasn't comfortable waiting that long and had Kyle examined again three months later. This time a sonogram test showed that Kyle had between 20 and 30 stones located in his kidneys. "No one could tell us for sure what was wrong with our son," says Brett. Kyle was diagnosed with everything from overproduction of uric acid to having a possible neurological disease. "Over a period of six months, doctors told my wife and me on two or three separate occasions that our son was going to die." Brett describes the experience as "an emotional roller coaster ride for all of us, including our extended families."

Debra Wattenberg and Brett Rosen became concerned when their son, Kyle, developed a high fever at six weeks of age. Kyle was eventually diagnosed with a rare form of hyperoxaluria, a metabolic disease that can lead to kidney stones and kidney failure. Researchers have identified genes thought to contribute to this condition, and hope to develop new therapies in the future. Such diseases exact a cost not only on the patient, but also on the patient's family. The family is hopeful that research will discover more effective approaches for combating this disease.

Desperate for answers, Debra and Brett decided to take action of their own. They stayed up late nights researching various diseases over the Internet, as well as talking to several different physicians. They were finally led to a specialist at the Mayo Clinic in Minnesota, who took a biopsy of Kyle's liver and diagnosed the then nine-month old boy with having a rare, or "undefined" form of hyperoxaluria, a genetic disease that results in the formation of kidney stones and that if left untreated could lead to kidney failure. Kyle, now 5 years old, is one of only a very small number of children worldwide diagnosed with this rare form of hyperoxaluria, and the medical community is still uncertain as to the cause or how to successfully treat it. While Kyle's future prognosis is unknown, what is certain is that time is critical for Kyle and other children with hyperoxaluria. Eventually, Kyle's kidneys will shut down and he will need either a kidney, liver or combined liver-kidney transplant, which, though considered valid treatments, come with considerable risk. To help children with this condition, Kyle's parents serve as active board members of the Oxalosis and Hyperoxaluria Foundation, which works to intensify research efforts.

The NIDDK is seeking to increase research in urinary tract stone disease, including hyperoxaluria. The NIDDK's Division of Kidney, Urologic and Hematologic Diseases has targeted research funds to develop strategies for:

- Correcting the genetic defect in oxalosis and hyperoxaluria;
- Developing new animal models to better study stone diseases; and
- Conducting research on families with a history of these kinds of diseases.

It is hoped that through NIDDK funding and support, new and innovative approaches for addressing the genetic causes and treatment for stone diseases will

be discovered and disseminated in time to help Kyle and other children who have or may develop the same conditions.

About Oxalosis and Hyperoxaluria

Hyperoxaluria has several forms, including primary hyperoxaluria type I and type II; unclassified, sometimes referred to as undefined hyperoxaluria; acquired hyperoxaluria; and absorptive or enteric hyperoxaluria. The common factor in all forms of hyperoxaluria is an excessive excretion of oxalate in the urine. Oxalate is normally eliminated by the body. When excess oxalic acid combines with calcium inside the kidneys, the combination results in kidney stones. Oxalosis occurs when the kidneys fail to eliminate calcium oxalate crystals from the body through the urine.

Children with hyperoxaluria suffer from continuous stone formation, which ultimately leads to kidney failure. As this kidney disease progresses, calcium oxalate is deposited in vital organs resulting in disease of the heart, bone, blood vessels, eyes, and finally progressing to end-stage oxalosis, which is potentially fatal.

Living with the Disease

The severity of hyperoxaluria varies widely, from a complete absence of symptoms and late development of kidney stones to an extremely serious and progressive disease. Most children are diagnosed between the ages of 10 and 20. Kyle, however, was one of the youngest children to be diagnosed with hyperoxaluria of an extremely rare type, and the constant formation of stones in his system has made the first five years of his young life extremely difficult for both him and his family.

At the age of one, for example, physicians removed a urinary stone, or calculus, from Kyle's urinary tract by inserting an endoscope in his penis. Kyle's stones also have been treated with extracorporeal shock wave lithotripsy (ESWL), where shock waves are used to break up the stones so that they can pass through the urinary system. Such treatment requires hospitalization and general anesthesia when treating young children. Despite the immediate relief each procedure brings, it doesn't prevent further stone development. Each year, Kyle experiences a couple of major stone episodes. "It's excruciating to watch your child continually go through these painful and risky procedures," says Kyle's father, Brett.

A primary treatment for hyperoxaluria is the consumption of large amounts of fluids, which helps keep the kidneys flushed and limits crystal formation. Because of this, Kyle drinks water throughout the day and has been trained to drink a bottle of water and a bottle of apple juice each night. As a result, this otherwise fully active little boy who swims, goes to day camp and pre-school, and functions perfectly well in society still wears diapers at night.

Many patients are also treated with large daily doses of Vitamin B6. Kyle, however, did not respond to Vitamin B6 supplements. He does take magnesium supplements, a diuretic twice daily, and a drug to help retard and prevent stone development. Despite the lack of medical evidence for diet restriction, he also is on an oxalate-reduced diet, which means no chocolate, berries, tomato sauce or ketchup. "When Kyle was three," says Debra, "it was difficult to send him to

HYPEROXALURIA FACTS

- **Primary hyperoxaluria is a genetic metabolic disease that results in excess oxalate in the urine, which forms kidney stones and may lead to kidney failure. It is most often diagnosed in children but may be diagnosed at any age.**
- **First signs of the disease may be blood in the urine, the painful passage of stones or urinary tract obstruction.**
- **To preserve kidney function, early diagnosis and treatment are essential.**
- **Although millions of Americans suffer kidney stones each year, hyperoxaluria is considered an "orphan disease" (suffered by fewer than 200,000 Americans).**
- **Little is known about the cause of the most common forms of calcium oxalate stone disease, and effective long-term preventive therapies and treatments are needed.**

friends' birthday parties and tell him he couldn't eat any pizza, chocolate cake or ice cream, or drink any cola-flavored soda. Now, at age five, Kyle will tell you 'I can't have chocolate or pizza with sauce because I have kidney stones.'"

The Future

Although urinary tract stone diseases, including all forms of hyperoxaluria, constitute a major health care burden for Americans, little is known about even the cause of the most common forms, and there are no long-term, effective preventive strategies. Recent findings, however, have opened new and exciting avenues for investigation into the cause and progression of kidney stones. Many scientists believe that genetic research is the best hope of finding a permanent cure for the various forms of hyperoxaluria. For example:

- The genes for primary hyperoxaluria types I and II have been identified, which will aid in diagnosing the disease.
- Studies of families with a history of kidney stones indicate that an inherited genetic defect is a likely cause of absorptive hypercalciuria (AH). Researchers have now identified a specific region on chromosome 1 that is associated with a severe form of this disease. It is hoped that further research may permit early diagnoses and possible prevention of kidney stones.

In addition, researchers have identified a new class of bacteria found in human urine and blood, which they believe may be related to levels of calcium in the body. They have also determined that oxalate synthesis is regulated by the metabolic state of the liver. Both of these findings may lead to the development of drugs to combat stone disease. In the meantime, Kyle and his parents hope and pray that a cure for his disease will be found before organ transplantation is necessary. "Finding a cure for calcium oxalate stone disorders will not only help Kyle and patients with primary hyperoxaluria, but the millions of Americans who suffer from kidney stones each year," says Brett.

Possible Vaccines for Urinary Tract Infections

Urinary tract infections (UTIs) are one of the most common medical problems in the U.S. A UTI produces symptoms such as painful, frequent urination. Bacterial infections may affect the kidneys, bladder, urethra, or ureters. More than 80 percent of UTIs are caused by the bacterium *Escherichia coli* (*E. coli*). Although antibiotics are frequently used to fight bacterial infections, an increasing number of antibiotic-resistant strains have forced scientists to search for other methods to treat common infections, including UTIs. NIDDK-supported researchers have uncovered new knowledge about how bacteria invade the urinary tract. These discoveries will help scientists develop new treatments for UTIs, such as vaccinations against the disease.

Vaccines work by presenting the body with an inactive form of a microorganism, or component of a microorganism, so that the body can make antibodies designed to attack and destroy it. After vaccination, the body is able to destroy invading microorganisms before they can do damage. Two different NIDDK-supported research groups have attempted to discover proteins that facilitate bacterial infection in order to develop vaccines against them. Both groups have described proteins vital to a bacterium's ability to bind to urinary tract cells.

Prior to invading bladder cells, bacteria attach to the cell surface using a threadlike extension called a pilus. The pilus is made of proteins that have a strong tendency to fold or bind to themselves or nearby proteins. Molecular chaperones are proteins that keep other proteins from binding or folding incorrectly. One research group demonstrated that bacterial pilus assembly requires a molecular chaperone from a family of proteins known as the PapD-like chaperones. Without the chaperone protein, the pilus doesn't form correctly and the bacteria are unable to attach to tissue. Preliminary tests suggest that both monkeys and mice vaccinated against the PapD-like chaperone proteins can resist bladder infections. A second research group identified a sticky protein at the end of the pilus called FimH, which helps the pilus attach to bladder cells. Three out of four monkeys vaccinated with FimH protein were able to fight off bladder infections even after *E. coli* bacteria were injected into their bladders. While neither vaccine has yet been tested in humans, these results suggest that vaccination against

the PapD-like chaperone proteins or the FimH protein of *E. coli* bacteria may enable humans to fight off UTIs.

Pilus-mediated attachment to the outside of urinary tract cells is well understood. However, photographs of rat and mouse bladders affected by UTIs show bacteria inside bladder cells. By getting inside cells, bacteria avoid being flushed out of the bladder by urine or attacked by the immune system. Identification of internalization mechanisms would provide an ideal pathway for prevention. Two groups of NIDDK-supported scientists have described ways that bacteria can get inside cells of the urinary system. One group reported evidence that the FimH protein on the surface of the *E. coli* pilus causes bladder cells to "ruffle up" on the surface. The "ruffles" surround and eventually "swallow" the bacteria. Researchers describe a detailed cascade of events inside the cell that occur after FimH attaches to the bladder cell surface. A second research team found receptors for FimH on the surface of immune-system cells called mast cells. Under normal conditions, one way that mast cells fight infection is by internalizing an invading substance and digesting it in cellular compartments called endosomes. In the case of *E. coli* bacteria, however, mast cells internalize the bacteria, yet never digest them within endosomes. The research group found evidence that tiny cave-like mast cell surface structures called caveolae contain receptors for the FimH protein. Mast cell caveolae bind to FimH on the bacterial pilus and enclose the bacteria in compact chambers inside the cell. The *E. coli* bacteria's use of caveolae to get inside mast cells is significant because the compact chambers formed by the caveolae do not fuse with endosomes. Thus, the bacteria again avoid destruction by "hiding" inside cells. With the expanding knowledge of how UTIs occur, it may be possible to develop drugs to interfere with FimH or caveolae proteins and prevent bacteria from getting inside bladder or mast cells of the urinary tract. The NIDDK supports basic research that will benefit not only a specific disease of concern to NIDDK, but will be useful for treating all diseases. In this instance, the general knowledge of bacterial invasion mechanisms will also be useful to prevent and treat many kinds of bacterial infections.

Barnhart MM, Pinkner JS, Soto GE, Sauer FG, Langermann S, Waksman G, Frieden C, and Hultgren SJ. PapD-like chaperones provide the missing information for folding of pilin proteins. *Proc Natl Acad Sci USA* 97(14):7709-7714, 2000.

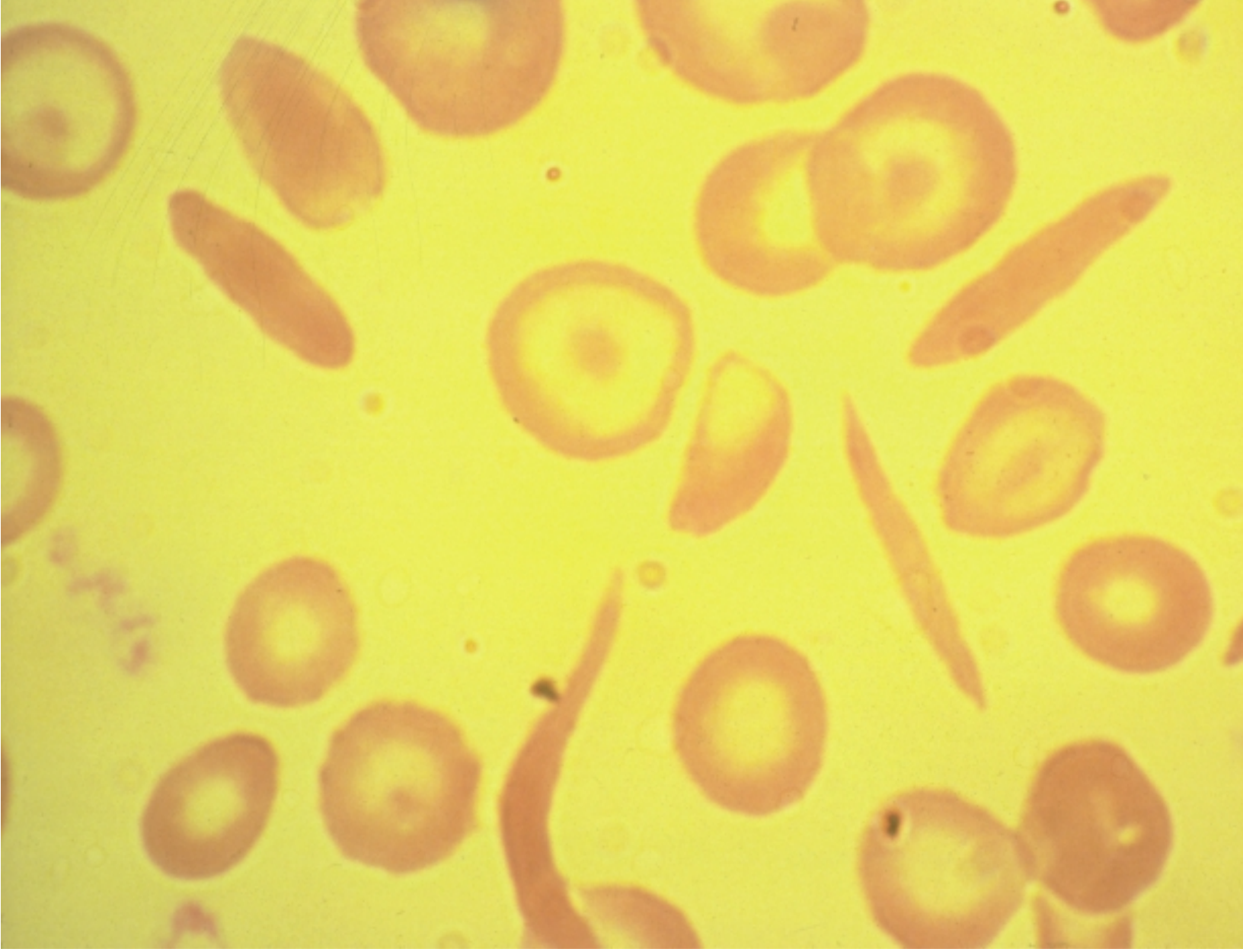
Langermann S, Mollby R, Burlein JE, Palaszynski SR, Auguste CG, DeFusco A, Strouse R, Schenerman MA, Hultgren SJ, Pinkner JS, Winberg J, Guldevall L, Soderhall M, Ishikawa K, Normark S, and Koenig S. Vaccination with FimH adhesin protects Cynomolgus monkeys from colonization and infection by uropathogenic *Escherichia coli*. *J Infect Dis* 181(2):774-778, 2000.

Martinez JJ, Mulvey MA, Schilling JD, Pinkner JS, and Hultgren SJ. Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. *EMBO J* 19(12):2803-2812, 2000.

Shin JS, Gao Z, and Abraham SN. Involvement of cellular caveolae in bacterial entry into mast cells. *Science* 289(5480):785-788, 2000.

Why a Cell's Shape Is Important: Sickle Cell Disease

What happens when the normal circular shape of a red blood cell is distorted to a curved shape? The result is sickle cell disease—a disease in which the sickle-shaped red blood cells cannot easily pass through blood vessels to bring life-giving oxygen to tissues throughout the body. Sickle cell disease affects approximately 72,000 people in the U.S., including nearly 1 in every 500 children of African American descent, and 1 in every 1,000-1,400 children of Hispanic American descent. Most patients do not survive beyond their mid-forties. The sickle-like shape of red blood cells in this disease is due to an error in the gene coding for hemoglobin, which is responsible for transporting oxygen throughout the body. Instead of being rounded, the hemoglobin is elongated and normally disc-shaped red blood cells become "sickle-shaped." Sickled cells are prone to rupture and do not transport oxygen efficiently. When patients lose numerous red blood cells and the tissues are deprived of oxygen, they develop a condition known as anemia. Symptoms of sickle cell anemia include paleness, shortness of breath, and fatigue. Ruptured and misshapen red blood cells also restrict blood supply by sticking together and blocking small blood vessels. Blocked blood vessels are directly responsible for many complications of the disease, including swollen hands and feet, organ and tissue damage—such as kidney failure—due to lack of blood supply, unpredictable recurrent pain episodes in organs or joints, eye problems, delayed growth in children, susceptibility to infections, and stroke. In an effort to improve treatment and quality of life for patients, the NIDDK is funding research aimed at developing new therapies for this disease.



In the photograph above, “sickle-shaped” cells are shown in a field that also contains normal, round cells. Individuals with sickle cell disease possess a mutation in one of the genes involved in the transport of oxygen by the blood. This mutation causes the red blood cells of patients to adopt a sickle-like shape, which impairs their ability to fit through tiny blood vessels and therefore deprives tissue of oxygen.
Photo: Dr. Griffin Rodgers, NIDDK.

Potential new therapies are often based on clinical observations. Doctors observed that patients whose blood contains high levels of fetal hemoglobin tend to have less severe symptoms. Laboratory experiments explained these observations by demonstrating that fetal hemoglobin prevents the abnormal hemoglobin from becoming elongated. As a result, patients with high concentrations of fetal hemoglobin have fewer sickled cells and suffer fewer symptoms due to blocked blood vessels. If doctors are to use this knowledge to treat patients, they must first know how much fetal hemoglobin is needed to alleviate symptoms. To answer this question, NIDDK-supported researchers mated mice expressing a human sickled hemoglobin gene with mice expressing human fetal hemoglobin. The offspring expressing both sickled and fetal hemoglobin had fewer sickled cells, less organ damage, and increased life spans as compared to mice expressing only the human sickled hemoglobin gene. The researchers determined that a nine percent increase in fetal hemoglobin levels was sufficient to correct many problems caused by sickle cell disease. These background investigations using mice have given researchers a better idea of how much fetal hemoglobin is likely to alleviate human symptoms. Potential treatments based on human gene transfer can now be tailored to increase fetal hemoglobin to this level.

Other treatments for sickle cell disease are aimed at improving circulation by either dilating the blood vessels or increasing the ability of sickled cells to bind oxygen. Previous research reported that the gas nitric oxide (NO) could both dilate blood vessels and increase the oxygen-binding ability of sickled cells. A recent NIDDK-supported study did not reach the same conclusions. Hemoglobin from patients who had inhaled NO did not demonstrate improved oxygen binding abilities, although the patients' red blood cells were able to transport NO to tiny blood vessels. This discovery provides an opportunity for improving circulation. If patients inhale NO at higher doses than used in this study, small blood vessels previously blocked by clumps of sickled cells may dilate, thereby alleviating many symptoms of the disease. The NIDDK continues to support studies directed toward finding improved treatments and toward increasing our understanding of the basic processes involved in blood cell generation and hemoglobin regulation. With an ever-expanding knowledge base, researchers hope to eliminate the pain and disability resulting from the genetic abnormality that causes the sickling of red blood cells.

Blouin MJ, Beauchemin H, Wright A, DePaepe M, Soreete M, Bleau AM, Nakamoto B, Ou CN, Stamatoyannopoulos G, and Trudel M. Genetic correction of sickle cell disease: Insights using transgenic mouse models. *Nat Med* 6(2):177-182, 2000.

Gladwin MT, Schechter AN, Shelhamer JH, Pannell LK, Conway DA, Hrinczenko BW, Nichols JS, Pease-Fye ME, Noguchi CT, Rodgers GP, and Ognibene FP. Inhaled nitric oxide augments nitric oxide transport on sickle cell hemoglobin without affecting oxygen affinity. *J Clin Invest* 104(7):937-945, 1999.

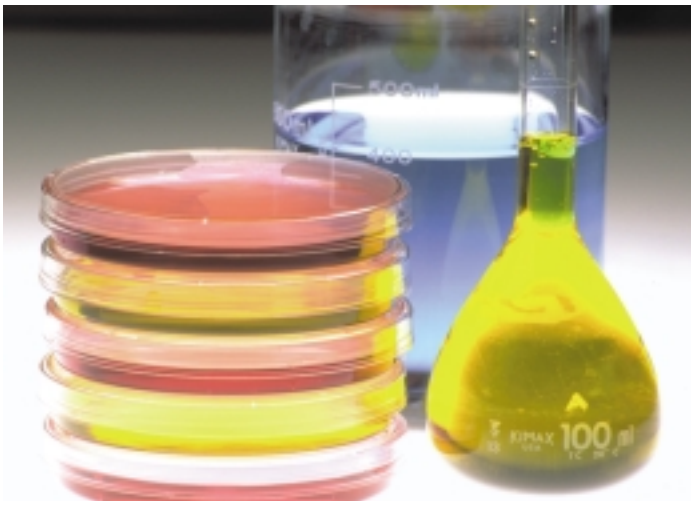


Photo: Mr. Richard Nowitz.

Iron Metabolism: A Key to Cooley's Anemia and Hemochromatosis

The amount of iron in the body must be strictly regulated. People whose bodies lack sufficient iron suffer from anemia, with symptoms such as pale skin, shortness of breath, and tiredness. On the other hand, people whose bodies store too much iron develop a disease called hemochromatosis. The most common symptom of hemochromatosis is joint pain. Left untreated, the excess iron causes joint and organ damage. Eventually, diseases such as diabetes, arthritis, liver disease, heart abnormalities, and thyroid deficiency develop. Iron levels can also become abnormally high when a person who is treated for severe anemia receives frequent blood transfusions. For example, patients suffering from Cooley's anemia (also termed thalassemia) have defective proteins in their red blood cells. As a result, the cells cannot transport enough oxygen to support the body's tissues. To correct this defect, regular transfusions are used to provide normal red blood cells with oxygen-transporting hemoglobin. However, iron from the transfused blood builds up in the body and can produce symptoms similar to those of untreated hemochromatosis. Transfusion, and drugs to remove excess iron, are expensive. Even more distressing, however, is that patients must endure painful injections on a daily basis. Because diseases involving iron regulation are common and often result in premature death, the NIDDK has placed a high priority on research that will lead to improved treatments. The NIDDK supports studies aimed at improving existing therapies and studies that increase fundamental understanding of how iron is regulated in the body. Both of these areas offer the potential for significantly improving the outlook of patients suffering from diseases of iron regulation.

Two separate NIDDK-supported studies have recently identified proteins responsible for iron regulation. One group of researchers identified a new type of iron receptor in mice that may be responsible for iron buildup in patients with hemochromatosis. Iron receptors bind to iron in the blood and provide a channel into cells whose membranes contain the receptor. Because the known iron receptor (transferrin receptor, or TfR) shuts itself down when exposed to excess iron, it was unclear just how iron continues to build up in hemochromatosis. The newly discovered iron transporter, called TfR2, is unable to shut down when exposed to excess iron and

thus may be responsible for storing excess iron in hemochromatosis patients. Potential treatments for iron regulation disorders may be able to deactivate this receptor and prevent the harmful buildup of iron in the body.

A second group of NIDDK-supported scientists identified an iron-transporting gene in zebrafish. The scientists examined zebrafish mutants whose blood lacked red coloring, indicating a deficiency in hemoglobin. Because hemoglobin formation requires iron, the scientists theorized that perhaps the mutant fish weren't making hemoglobin because they lacked iron in their blood. To test this theory, they injected iron solution into the blood of mutant fish. This treatment resulted in apparently normal blood cell coloration and normal levels of hemoglobin in red blood cells, which suggested that the fish do indeed have blood iron deficiencies. By comparing normal zebrafish DNA to DNA taken from zebrafish mutants, they discovered an error in a presumed iron-transporting gene, which they called *ferroportin1*. Zebrafish *ferroportin1* was expressed in areas of high iron transportation, as were ferroportin-like genes in mice and humans. Scientists can now use the knowledge of these new iron regulation proteins to develop potential treatments for diseases of iron regulation.

Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, Moynihan J, Paw BH, Drejer A, Barut B, Zapata A, Law TC, Brugnara C, Lux SE, Pinkus GS, Pinkus JL, Kingsley PD, Palis J, Fleming MD, Andrews NC, and Zon LI. Positional cloning of zebrafish *ferroportin1* identifies a conserved vertebrate iron exporter. *Nature* 403(6771):776-781, 2000.

Fleming RE, Migas MC, Holden CC, Waheed A, Britton RS, Tomatsu S, Bacon BR, and Sly WS. Transferrin receptor 2: Continued expression in mouse liver in the face of iron overload and in hereditary hemochromatosis. *Proc Natl Acad Sci USA* 97(5):2214-2219, 2000.

Photo: Mr. Richard Nowitz.

Model Systems – The Zebrafish

The tiny zebrafish, appropriately named for its black and white stripes, is often seen darting about among the colorful stones and greenery of home aquariums. Now, it is also seen in research laboratories. The zebrafish is one of the many animal models that have become an essential tool of biomedical research. These models provide scientists with a vehicle to explore the design and function of proteins and other molecules that are responsible for life and that are perturbed by disease.

The zebrafish joins the ranks of other useful animal models, including the fruit fly, the worm, and the mouse. An ideal animal model will:

- Reproduce quickly and in large numbers;
- Require low maintenance, at a reasonable cost;
- Have a comparable biological make-up to humans;
- Be able to provide a plethora of mutants for research studies.

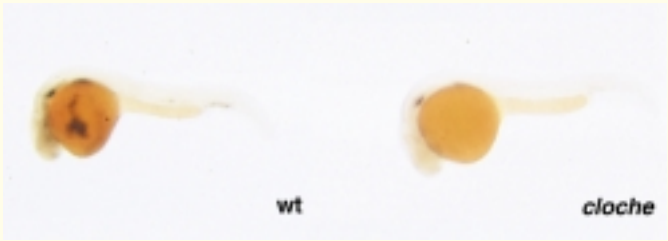
One of the earliest and most useful models was the fruit fly, which has been employed widely for the study of classical genetics and for learning about the structure and function of biological systems. The fruit fly grew quickly in popularity as a tool for developmental genetics during the 1970s, after European researchers were able to use a chemical to achieve random mutations of the DNA of thousands of flies. When offspring of the treated flies were screened for abnormalities, they produced an array of mutants that would subsequently lend major insights into the developmental processes of the fly. Because many of these biological processes were found to be universal, the flies provided important clues about biological processes in other organisms, including humans. The importance of this work was recognized with the award of a Nobel Prize in 1995.

The zebrafish: A model system for understanding embryo development and disease. Photo: Dr. Leonard I. Zon, Howard Hughes Medical Institute, Children's Hospital, Boston, Massachusetts.

Similar in research utility to the fruit fly, the zebrafish is now emerging as a model of choice for many research studies. While it has many attributes in common with the fruit fly, the zebrafish has the advantage of being a vertebrate, like man. Thus, insights gained from studies of the zebrafish are more likely to be relevant to humans. In addition, because the fish embryos are transparent and develop outside of the mother, researchers can visually track their development with ease, and identify mutants as they arise. The zebrafish is also small, but exceptionally prolific in reproduction over short periods of time. Only an inch long, the zebrafish produces over 200 eggs per week. Thus, laboratories can readily house thousands or even hundreds of thousands of zebrafish.

The researchers who screened thousands of fruit flies have used the same technique for mutating zebrafish, with impressive results. The screens yielded over 2,000 mutant zebrafish. Exposing the zebrafish to a chemical mutagen results in discrete changes, or point mutations, in the DNA of the zebrafish sperm. When these fish mate, the changes in the sperm DNA are passed on to the offspring. If the mutant offspring are mated, the next generation will produce zebrafish with many mutations.

Because the zebrafish develops outside the mother, the clear embryo provides a window through which the formation of the organs and organ systems can be



The zebrafish embryos above show expression of the blood specific marker NFE2. This marker, shown in black, detects expression of blood in the wild type embryo (wt, left). Expression is not present in the mutant cloche, which has no blood or blood vessels (right). Photos: Dr. Leonard I. Zon, Howard Hughes Medical Institute, Children's Hospital, Boston, Massachusetts.

viewed under a microscope. Enhancing this exceptional visualization is the rapid speed at which development occurs. The embryo heart is formed and begins beating approximately 24 hours after fertilization. At that time, the blood begins circulating and it is possible to observe fish whose mutations affect the vascular system. For example, pale colored blood identifies a fish that is suffering from anemia. The names of wines have been used for many fish that have such blood disorders. Some fish are called chardonnay and sauternes because their blood has decreased levels of hemoglobin, the molecule that is responsible for carrying oxygen in red blood cells and giving the cells their red color.

Fifty mutants have been found that affect the development of blood cells. The sauternes mutant was first identified in embryos by the pale color of its blood 33 hours after fertilization had taken place. Scientists were able to isolate the sauternes gene and identify it as the gene coding for the enzyme ALAS2, a protein essential in hemoglobin synthesis. Defects in the human ALAS2 gene cause a disease known as congenital sideroblasic anemia. The sauternes zebrafish now provides the first animal model for the study of this disease.

Another zebrafish with pale colored blood due to anemia is the weissherbst mutant. Scientists have recently identified the gene responsible for the underlying anemia. Iron is the element that binds oxygen within the hemoglobin molecule. Defects in iron metabolism can result in either diseases of excess iron, such as hemochromatosis, or diseases of iron deficiency, the anemias. The weissherbst gene, *ferroportin1*, was characterized as a transmembrane protein that is required for iron transport in the zebrafish. It is likely that this zebrafish will prove valuable as a model for accumulating new knowledge about such diseases.

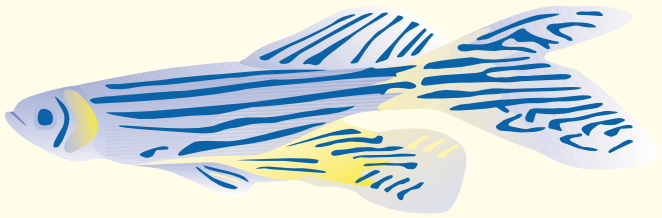
The zebrafish also demonstrates the exceptional value of animal models that have genes highly analogous to those in humans, and in which discoveries may therefore have a ready human parallel. This type of similarity exists between zebrafish and humans, known as conserved chromosomal synteny. To date, genes that have been located on the zebrafish chromosome 9 have also been found on the human chromosome 2. Conversely, if a human gene is identified, it is likely that the analogous zebrafish gene can be located in the same manner, and provide a way to gain insights about the human genetic disease. Once an animal model is identified for a particular disease, a second-generation screen can be performed to correct or negate the disease in the animal. These mutants might then point the way to new treatments for humans.

The NIH is a major force in the progress now under way in zebrafish research, with the NIDDK playing a key role in scientific leadership of the initiative. The community of zebrafish researchers has promoted the use of the zebrafish as a model organism for the study of vertebrate development and disease. In response to recommendations from this community, the Director of the NIH established the Trans-NIH Zebrafish Coordinating Committee, which is composed of representatives from 16 NIH Institutes and Centers and co-chaired by representatives from the NIDDK and National Institute of Child Health and Human Development. A Genomic Resources Initiative was subsequently undertaken for positional and candidate-based cloning approaches needed to isolate the genes of the many zebrafish mutants that could be informative with regard to developmental biology. Most recently, a trans-NIH initiative was announced to increase the number of small pieces of expressed genes in the zebrafish called Expressed Sequence Tags or ESTs. Analysis of the ESTs is expected to identify over 30,000 unique zebrafish genes that can be genetically mapped using a successful technique known as radiation hybrid mapping. The NIDDK is also bolstering support of its current grantees who are involved in EST research. Another initiative is

under way to encourage the development of improved or novel methods for a form of sophisticated genetic screening, termed mutagenesis screening, in the zebrafish in order to identify and characterize genes important to diseases within the NIDDK mission. During the past few years, an explosion of scientific advances has occurred based on the zebrafish as a significant vertebrate model. The NIDDK continues to be key to this progress by keeping abreast of the needs and opportunities of the zebrafish research community and by supporting the development and use of the zebrafish and other model systems for solving the genetic and developmental puzzles of many diseases. The zebrafish model is now being extended to other developmental processes and diseases of research interest to the NIDDK including the development of the pancreas and its insulin-producing islets, the gallbladder and gut, and the kidney.

Brownlie A, Donovan A, Pratt SJ, Paw BH, Oates AC, Brugnara C, Witkowska HE, Sassa S, and Zon LI. Positional cloning of the zebrafish *sauternes* gene: A model for congenital sideroblastic anaemia. *Nat Genet* 20(3):244-250, 1998.

Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, Moynihan J, Paw BH, Drejer A, Barut B, Zapata A, Law TC, Brugnara C, Lux SE, Pinkus GS, Pinkus JL, Kingsley PD, Palis J, Fleming MD, Andrews NC, and Zon LI. Positional cloning of zebrafish *ferroportin1* identifies a conserved vertebrate iron exporter. *Nature* 403(6771):776-781, 2000.



http://www.nih.gov/science/models/

Animal models are critical to scientists' understanding of development and disease. The zebrafish *Danio rerio* is an important vertebrate model described on NIH's website: Model Organisms for Biomedical Research (http://www.nih.gov/science/models/).

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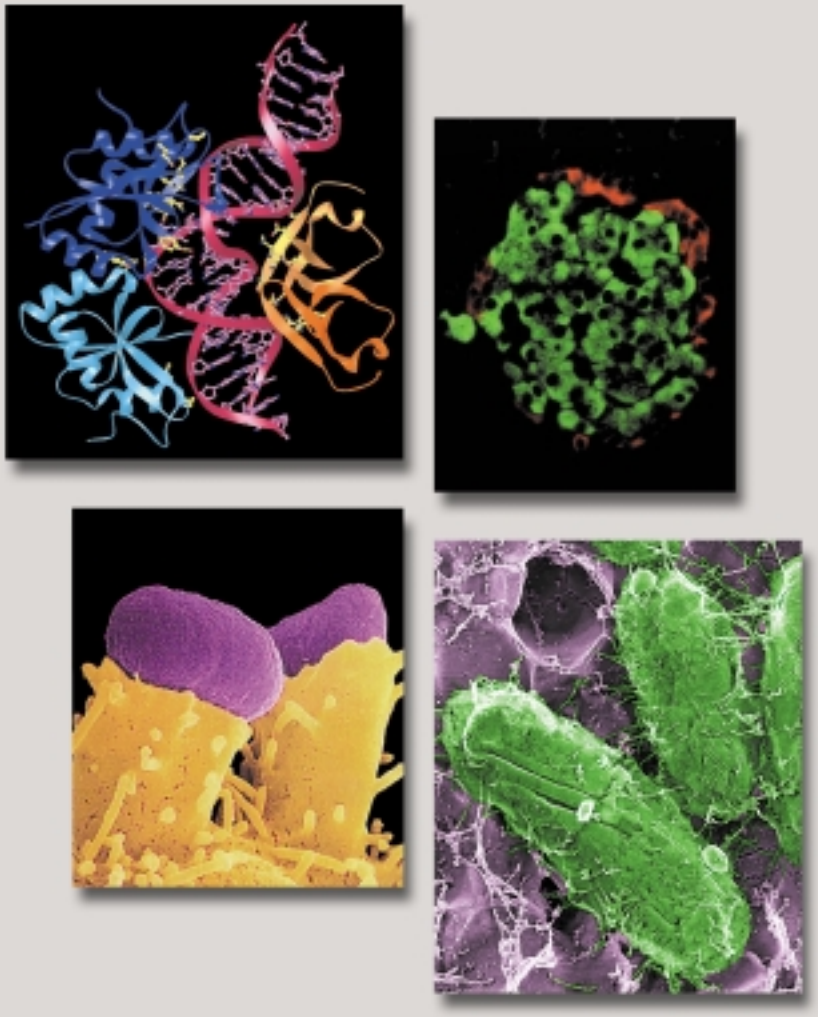

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