Thalassemias: Models of Genetic Diseases

For the first time, the molecular basis of a class of genetic diseases is known in detail

About 2 years ago, bone marrow samples from three patients with β^+ thalassemia, a rare form of anemia, were sent to Jeffrey Ross, a medical researcher at the University of Wisconsin who is studying the molecular basis of this disease. Although all three patients had the same genetic disorder, their anemias differed in severity. Two of the patients were seriously affected, requiring monthly transfusions to maintain hemoglobin concentrations within 75 percent of normal. The third had a milder anemia and did not require transfusions.

This sort of variation in clinical symptoms is typical of genetic diseases, according to David Nathan of Harvard Medical School and Children's Hospital in Boston, who is an authority on genetic diseases, particularly thalassemias. But only in the case of the thalassemias are investigators beginning to discover why. "From the point of view of both understanding and treating genetic diseases, I can't think of a better model than the thalassemias," says Nathan. The work also has a theoretical payoff. It is a first step toward learning how a group of human genes are regulated.

Thalassemia is a catchall term for inherited blood diseases in which a globin protein is missing entirely or is produced in diminished amounts. Adult hemoglobin consists of four globin protein subunits, two α globins and two β globins. When insufficient quantities of either α or β globin are made, too little hemoglobin is produced and patients are anemic. In addition to the adult globin genes, there are also two fetal genes, called γ, which substitute for the β genes during fetal life. When babies are born, their γ genes are irreversibly turned off in most blood cells and their β genes are turned on. A few cells, however, continue to make γ globin and so 1 to 2 percent of the total hemoglobin adults make is fetal hemoglobin.

In this country, thalassemias are rare—fewer than 1000 people are afflicted. The diseases are fairly common, however, in the Mediterranean area and in the Middle and Far East. In Cyprus, for example, five in every 1000 newborns inherit a defective globin gene from each parent and are severely anemic. It is not clear why thalassemia is so prevalent in these geographic areas, although it has been postulated that the genes may confer some resistance to malaria, as the gene for sickle cell anemia does. But unlike the resistance conferred by the sickle cell gene, malaria resistance due to thalassemia genes is by no means demonstrated.

The main reason why thalassemias are so intensively studied in this country, however, is not that the diseases are a major public health problem but that they are ideal for analysis with newly developed tools of molecular biology. Immature red blood cells are easily obtained and they synthesize hemoglobin almost exclusively—95 percent of the proteins they make are globins, whereas in most cells, no particular protein is more than 1 percent of the total. When globin proteins are made in enormous quantities, so are globin messenger RNA's (mRNA's), the copies of globin genes that are translated into proteins in the cell cytoplasm. It is thus fairly easy to isolate these mRNA's and make from them complementary DNA molecules, which bind exclusively to globin genes and can serve as molecular probes for the genes.

Recent technical advances have made it possible for researchers to use these probes to pick out, clone, and sequence normal and abnormal globin genes even though these genes are only one part in 10 million of a cell's total DNA. Another advantage in studying thalassemias is that the inheritance patterns and clinical symptoms of the diseases have been extensively documented. As a result, medical investigators often know a great deal about a patient's disease before they begin to analyze it by isolating, cloning, and sequencing the genes.

What researchers have learned in the past few years is that there are many causes of thalassemias. Globin genes can be completely or partially deleted. They can have mutations that affect their regulation or mutations that shut off globin protein synthesis. The variety of causes of thalassemias accounts for the diverse clinical manifestations of the diseases. Other genetic diseases probably are similarly heterogeneous, researchers believe.

Among the most intriguing of the thalassemias are the β^+ thalassemias. Patients with these thalassemias make normal β globin but in abnormally small quantities. As the patients Ross studied illustrate, their anemias can range from mild to severe, depending in part on how much β globin they make. What seems to be happening is that the regulation of β globin genes is disturbed. The β^+ thalassemias may allow researchers to determine precisely how globin, and possibly other genes as well, are normally regulated, since an understanding of what goes wrong with genes frequently leads to an understanding of how genes function.

Three groups of researchers recently found that the regulatory defect in β^+ thalassemias is tied to a major and surprising finding about the organization of the genes of higher organisms. In the past few years, molecular biologists discovered that the genes of higher organisms are in pieces on the DNA, separated by large DNA segments of un-

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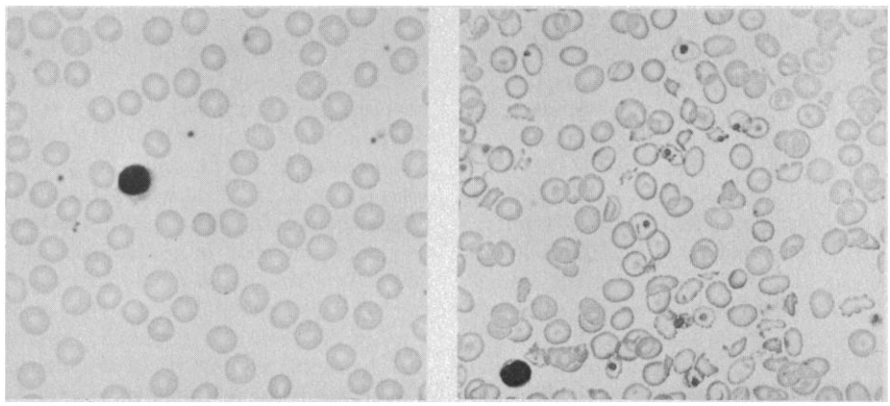
known function, called intervening sequences. When a gene is expressed, the entire DNA segment, including the intervening sequences, is transcribed into RNA. Then these large strands of RNA are processed into mRNA's by enzymes that cleave the copies of the intervening sequences and splice together the copies of the gene.

Since the intervening sequences were discovered, researchers have postulated that mutations in these sequences cause inherited diseases. Now it seems that B^+ thalassemias may be the first examples of diseases caused by such mutations.

Working independently, Ross, Arthur Nienhuis, and Judith Kantor of the National Heart, Lung, and Blood Institute, and Bernard Forget and Edward Benz, Jr., of Yale University have found that large RNA copies of the β globin gene and its intervening sequences pile up in β^+ thalassemic cells but that few β globin mRNA's are produced and so few β globin proteins are made. Ross has also demonstrated that the difficulty is in the processing of these long RNA's into β globin mRNA's. Presumably, there are mutations in the intervening sequences of β globin genes at sites recognized by enzymes that cut and splice the long RNA's. As a result, splicing is inefficient.

Evidence for this scenario comes from Richard Spritz at the laboratories of Forget and Sherman Weissman of Yale. Spritz, Forget, and Weissman cloned and sequenced a β globin gene region from a patient with β^+ thalassemia and compared it to a normal β globin gene region sequenced by Tom Maniatis, Richard Lawn, and Catherine O'Connell at the California Institute of Technology together with Argiris Efstradiadis at Harvard Medical School. There were no abnormalities in the DNA region flanking the gene, but there was one nucleotide difference in the smaller intervening sequence. This change creates what looks like a new splicing signal and so may account for the abnormal RNA processing.

Intervening sequences do not code for proteins and they seem to be normally far more variable than gene sequences, which do code for proteins. It is highly likely that variations may be found in intervening sequences of β^+ thalassemia patients that are of no biological significance. Many more intervening sequences from β^+ thalassemia patients will have to be analyzed before anyone can decide which regions of these sequences are crucial for RNA processing and why. Most likely, a number of different mutations can affect processing, but to various degrees. This would explain why some patients are more severely anemic than others. "We now have 5 patients, each of whom has inefficient processing," says Ross. "But the efficiency of processing and the RNA splicing patterns differ."



Normal and diseased blood cells

The cells on the left are from a normal individual. Those on the right are from a patient with a severe form of thalassemia. [Source: Arthur Nienhuis, National Heart, Lung, and Blood Institute]

Patients with another form of β thalassemia, β^0 thalassemia, may have intervening sequence mutations that completely prevent splicing and thus mRNA production. Several laboratories have isolated β^0 genes from patients and are investigating this possibility. But this disease is very heterogeneous and may be due to gene deletions or to errors in transcription or premature termination of protein synthesis as well as to errors in processing.

In the few cases where the causes of β^0 thalassemia are known, the causes differ greatly. For example, some patients were found by Stuart Orkin of Children's Hospital in Boston and, independently, by Richard Flavell of the National Institute for Medical Research in Mill Hill, London, to have partially deleted β globin genes. And one patient, studied by Y. W. Kan of the University of California at San Francisco, has a nonsense mutation in his β globin gene. Such a mutation changes a sequence of three nucleotides that normally code for an amino acid into a sequence that is a stop signal for protein synthesis. The β globin gene of Kan's patient is transcribed and processed into mRNA, but β globin protein synthesis is prematurely terminated.

One of the hopes of hematologists is to find ways to treat patients with β thalassemias by turning on their fetal globin genes. There are two thalassemias in which the β globin gene is deleted, the fetal γ globin genes are never turned off, and fetal hemoglobin is produced in adult life. In one of them, hereditary persistence of fetal hemoglobin, there are no clinical symptoms—the patients are not anemic.

A major problem with trying to find ways to turn on fetal globin genes is that it is not yet clear what turns them off in the first place. One possibility is that the fetal genes are turned off when a minor adult gene, called δ, is activated. The δ and β globins are very similar—they differ in only 10 amino acids and δ globin acts like β globin in forming hemoglobin. But, for unknown reasons, 40 β globin proteins are produced for every δ globin protein.

Hereditary persistence of fetal hemoglobin occurs when both the δ and β globin genes are deleted. Then the fetal γ globin genes are fully expressed. Another disorder, $\delta\beta$ thalassemia, occurs when the β gene is completely deleted and the δ gene is partially deleted. Patients with $\delta\beta$ thalassemia make fetal hemoglobin but not in sufficient quantities, so they are anemic. "Something in the δ region suppresses γ globin," says Arthur Bank of Columbia University.

But other explanations for the turning on of γ genes when the δ gene is deleted are also possible. One favored by Flavell is that the deletion of the δ gene changes the conformation of the DNA region containing the γ genes, making these genes more accessible. Harold Weintraub of the Hutchinson Cancer Center in Seattle has some evidence that, in chickens, the DNA region containing embryo and adult β globin genes is more accessible during embryonic life. He proposes that the region containing the embryo and adult globin genes is looped out in embryo cells. The embryo globin genes, he suggests, are at the base of the loop. He speculates that when the embryo genes switch off, the loop becomes

smaller so that they are no longer part of it and therefore are inaccessible to enzymes that copy the genes into RNA.

Not unexpectedly, since adult hemoglobin consists of two β globin and two α globin proteins, thalassemias can result from deficiencies in α as well as β globin synthesis. The α thalassemias, Bank explains, have been considered "relatively boring," because they result mostly from gene deletions rather than deficiencies in gene regulation. But recently, several laboratories have revived interest in these disorders by discovering how the deletions seem to occur. It is postulated that they occur by a form of gene shuffling called unequal crossing-over that can take place whenever sequences are repeated on DNA.

Unlike the β globin genes, the α globin genes are tandemly repeated. There are two α globin genes on each copy of chromosome 16, so each person normally inherits four such genes—and in abnormal situations from one to all four can be deleted. Kan found that, as more α genes are deleted, the corresponding α thalassemias vary in severity. Most of the α thalassemia carriers who have clinically normal hemoglobin production have a deletion of one α globin gene. When two α genes are deleted, Kan discovered, some anemia occurs. When three are deleted, the anemia is severe, and when all four are deleted, the anemia is fatal and affected fetuses are stillborn.

Stuart Orkin recently studied the DNA sequences deleted in individuals who seemed to have one α globin gene missing. He discovered that the left-hand portion of the first α gene was present and the right-hand portion of the second α gene was present. But, Orkin says, "it looked as though the middle was taken out" of the two-gene sequence. In effect, the equivalent of one α gene was deleted. He proposed that one way to account for such a deletion pattern is to suppose that unequal crossing-over occurs. Kan subsequently found another sort of α globin gene deletion in which the middle of the two-gene sequence was gone, but the deletion was shifted somewhat to the right of the one that Orkin discovered. This deletion, too, could be accounted for by unequal crossing-over. If unequal crossing-over causes the deletions of α globin genes, some individuals should have three α globin genes on a chromosome whereas others should have one. Kan and David Weatherall of Oxford University recently, and independently, reported finding people with three α globin genes.

Further evidence for this mechanism was obtained by Joyce Laver, James Shen, and Maniatis. They put α globin genes on bacterial viruses and allowed the viruses to multiply in bacteria. He and his colleagues found that two kinds of α globin gene deletions occurred by unequal crossing-over and that these are the same deletions that occur in α thalassemias.

In the past few years, then, researchers have been able to describe the molecular basis of the thalassemias—an entire class of genetic disorders—in a detail previously unimaginable. "To me, that is intellectually satisfying," says Nathan.

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Additional Reading

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NMR Opens a New Window into the Body

The use of nuclear magnetic resonance for medical diagnosis hovers on the brink of practical application

The goal is far simpler to describe than to achieve. All that is wanted is a completely noninvasive—and risk-free—method for seeing what is happening inside the human body.

No technique that is currently available fits this description. Even devices that require x-rays, such as the well-known CAT (computer-assisted tomography) scanners, are invasive in the sense that they expose the patient to varying doses of ionizing radiation, which has been linked in animal studies to an increased risk of cancer and birth defects.

A new approach, in which nuclear magnetic resonance (NMR) signals are used to construct two- and three-dimensional images of portions of the human body, may provide what appears to be at least a very-low-risk, if not a risk-free, diagnostic technique. Within the past 6 months to a year, researchers have begun to produce NMR images in 2 or 3 minutes that are able to distinguish features with dimensions as small as 2 millimeters, a marked improvement over the situation just 3 or 4 years ago, when the first images began to appear. At that time, image production required as much as 30 to 40 minutes, far too long to be practical for medical diagnosis; furthermore, the resolution was poor.

With the recent improvements in hand, investigators are just beginning to move from laboratory studies, usually with themselves as the subjects, to clinical evaluation of NMR imaging in patients with known pathological conditions. One potential application is in cancer diagnosis; additional applications, such as heart attack detection, may be possible even if they are not imminent.

Chemists have used NMR for almost three decades as a tool for working out the molecular structure of pure, homogeneous samples. Suggestions that NMR might also be applied to the far more complex problem of producing body images are more recent, dating back only about 10 years.

There is some controversy about who should get credit for the development of the new NMR methods. Raymond Damadian, who is affiliated with the Downstate Medical Center of the State University of New York (SUNY) claims that the idea originated with him, but so does Paul Lauterbur, who is at SUNY's Stony Brook campus. Some of the investigators now actively pursuing NMR imaging research attribute their own interest to the work of Lauterbur. For example, Waldo Hinshaw of Massachusetts General Hospital says, "I consider Lauterbur to be the father of the field; I think the literature shows that the idea originated with him." Damadian published the first paper, however.

It appeared 9 years ago in *Science* (19 March 1971, p. 1151), and presented evidence indicating that cancerous and nor-

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