The Connective Tissue Perspective

Molecular biology is linking a variety of diseases to specific abnormalities in the components and control of connective tissue

BY JULIE ANN MILLER

Connective tissue supports the body and holds the parts together. It stores and transports materials and repairs injury damage. Bone, cartilage, fat and even blood are prominent examples of connective tissue.

When a genetic defect interferes with normal connective tissue structure, the consequences are complex and often fatal. Excessive height, fragile bones and skin, hyperextendable joints, deafness, skeletal deformities and cardiovascular abnormalities are among the symptoms of inherited connective tissue diseases.

Diversity of inherited disorders of connective tissue has long been recognized. As more thorough analysis has become possible, physicians have repeatedly reclassified and subdivided the grouping of these rare syndromes. In a symposium on connective tissue at the recent meeting in Washington of the American Association for the Advancement of Science, Victor A. McKusick of Johns Hopkins University pointed out the consistent thickening of each new edition of the standard reference in this area, his book *Heritable Disorders of Connective Tissue.*

Recently investigators have begun applying new molecular biological techniques to normal production of connective tissue and to its disorders. The heterogeneity of the diseases seems to reflect a complex biochemical scheme for connective tissue formation and its control. Understanding gained from these studies offers new concepts for application to more widespread chronic diseases involving connective tissue.

Some connective tissue disorders are due to abnormalities in collagen, the most abundant of all proteins in mammals. This protein, found in all multicellular organisms, is the major fibrous element of skin, bone, tendon, cartilage and teeth, and represents a quarter of the body's protein.

The basic structural unit of collagen is the longest protein known—a triple helix, 3,000 angstroms long and only 15 angstroms across. The five known types of human collagen differ in the amino acid sequence of the three polypeptide chains that go into the triple helix. In some types of collagen the three chains of the helix are identical; in others the molecule has two chains of one sequence and one chain of another.

Although a triple helix seems like a simple enough structure, scientists are finding that collagen is made by a complicated series of events. The individual polypeptide chains are synthesized as longer precursors and then decorated with hydroxyl groups and sugars. A triple helix, called procollagen, forms and is secreted into the space between cells. Specific enzymes then clip off the stretches of polypeptide that extend from the helix at each end.

The rate of collagen synthesis is tightly controlled, probably at a variety of stages. Ronald Crystal of the National Heart, Lung and Blood Institute proposes a special regulatory step. He has been examining procollagen production from skin fibroblast cells and reports that in laboratory culture, each cell quite consistently makes 500,000 peptide chains to go into procollagen each hour.

Crystal argues that a degradation process has an important regulatory role in these cells. If a cell makes defective, nonhelical collagen, the concentration of the small messenger molecule cyclic AMP rises and type I collagen, the major product of fibroblasts, is degraded. But the enzymes that destroy defective procollagen are active most of the time, so they also may control normal procollagen output of a cell. Crystal suggests, for example, that natural signals, such as hormones, that increase cyclic AMP influence the cells by increasing degradation and thus decreasing production of type I collagen.

The complex scheme for collagen production allows plenty of opportunities for problems. The collagen gene may be defective; there may be an abnormality in regulation of subunit production or in processing of the chains. Darwin J. Prockop of the College of Medicine and Dentistry of New Jersey-Rutgers Medical School describes recent progress in detecting mutations underlying specific cases of hereditary disease. He has characterized the differences between collagen production of normal cells and those from patients with connective tissue disease.

The new work depends on DNA analysis sensitive enough to detect the mutations of interest. Large insertions and deletions of genetic material are relatively easy to pick up, but determining the exact nucleotide sequence of the gene of so large a protein to find the cause of a single altered amino acid is a "horrendous task," Prockop admits. (The collagen peptide chain is ten times longer than the peptide chains of globins, molecules that were among the first medically important genes to be sequenced [SN: 12/20 & 27/80, p. 396].) However, the sequence of one normal human collagen chain—alpha 1 of type I collagen—recently was determined by Prockop and colleagues Francesco Ramirez, Jeanne Myers and Mon-Li Chu. They also have analyzed this gene in a patient with an inherited bone disease. They found a difference, but the question remains whether this change is actually the cause of the disease. Prockop and colleagues are now investigating whether the patient's altered polypeptide chain can fold into a suitable triple helix, whether it is secreted, whether it is degraded and whether it can be part of a normal collagen fibril.

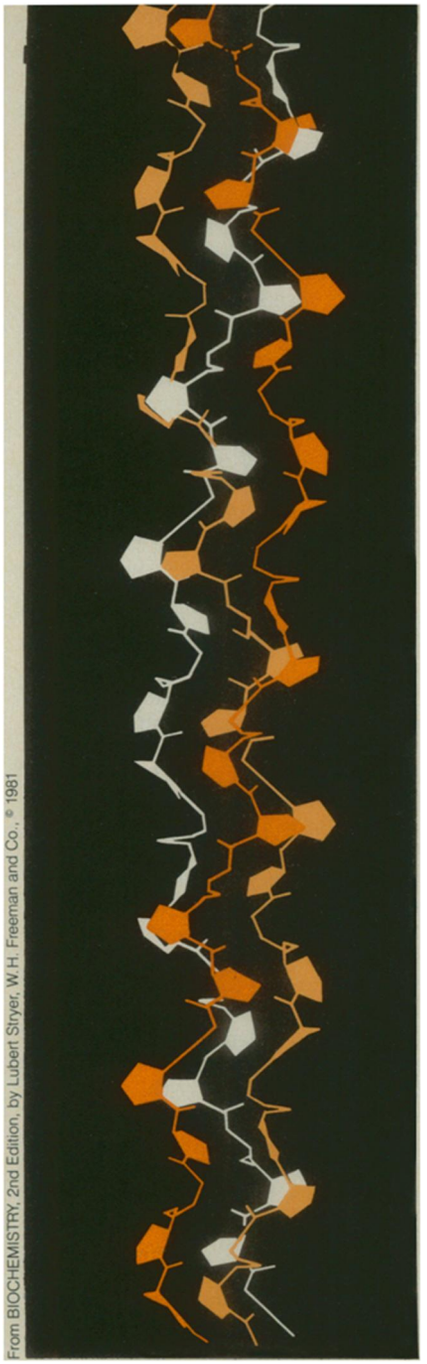
Prockop describes several other cases of connective tissue disorders in which the defect has been worked out. Osteogenesis imperfecta is a heterogeneous group of hereditary disorders affecting 20,000 to 30,000 people in the United States. It is characterized by fragility of bones, and in the most severe forms the fetus dies before birth. In moderate forms, repeated fractures cause bone abnormalities and there may be blue "whites-of-the-eyes," opalescent teeth, hearing impairment and thin, translucent skin.

Many forms of osteogenesis imperfecta seem to involve changes in the structure or synthesis of collagen. Prockop and colleagues examined collagen from fibroblasts of a male patient who had multiple fractures. They found the collagen contained excess amounts of the sugar mannose attached to one end of procollagen type I, probably due to a genetically determined change in amino acid sequence. The patient's protein tended to form aggregates, so the investigators surmise that less may be available for assembly of collagen fibrils. This shortage could explain the clinical manifestations of the disease.

In another case, fibroblasts were taken from a child with moderate brittle bone disease. The cells secreted type I procollagen containing only the polypeptide chain alpha-1, instead of the normal two chains of alpha-1 and one chain of alpha-2. Prockop recently determined that alpha-2 chains are made within the cells of this patient, but for some reason are not incorporated into the triple helix.

Prockop also describes fibroblasts taken from a stillborn baby with osteogenesis imperfecta. The cells produced equal amounts of normal and shortened alpha-1 subunits. The cells contained both normal and shortened alpha-1 messenger RNA, so Prockop and colleagues conclude that the baby may have had either one shortened alpha-1 gene or a genetic defect

Right: In osteogenesis imperfecta, repeated fractures cause bone abnormalities.
This victim is 20 years old, but less than 4 feet tall.

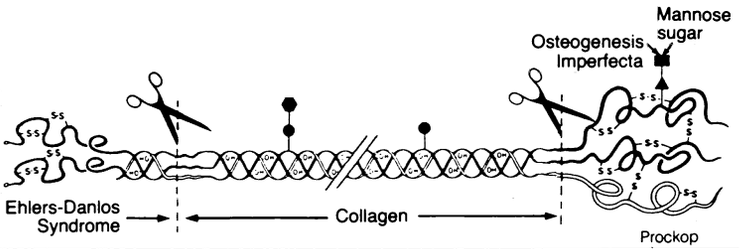


From BIOCHEMISTRY, 2nd Edition, by Lubert Stryer, W.H. Freeman and Co., © 1981

Above: Triple-stranded helix of collagen is decorated with sugar groups.

Osteogenesis Imperfecta Foundation, Inc.

Right: Inherited abnormality at one end of procollagen molecule results in one form of Ehlers-Danlos syndrome; excess sugars at the other end result in a case of osteogenesis imperfecta.



Collagen & Cancer: Breaking the Barriers

Connective tissue, as an organizational element of the body, provides barriers to the movement of normal cells. During cancer metastasis, however, cells break away from the primary tumor and traverse the barriers to colonize distant organs. Lance A. Liotta of the National Cancer Institute describes the specific biochemical interactions that allow the cancer cells to burrow through the connective tissue barriers of the basement membranes (structures that separate layers of cells) and intercellular matrix (material between cells in a tissue). "Metastasis is an active invasion, not a passive falling of cells off a tumor," Liotta stresses.

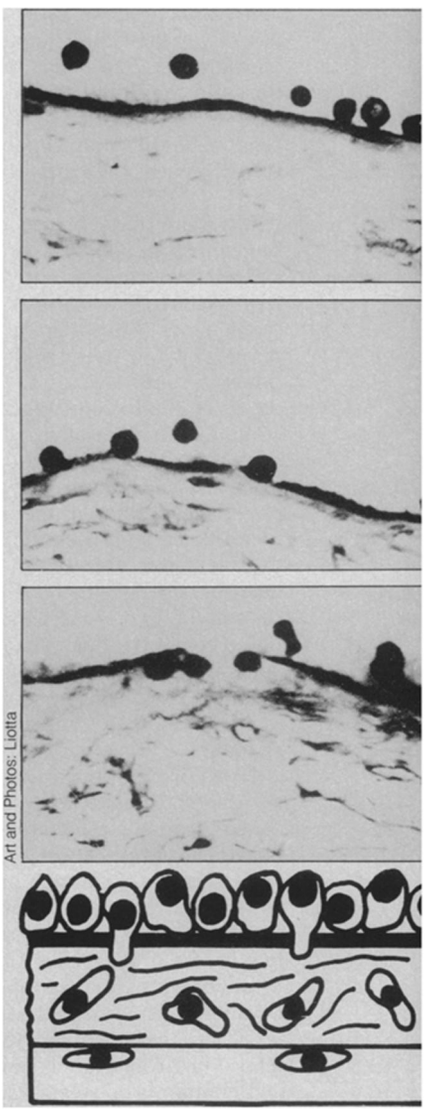
The first step in crossing connective tissue is attachment to collagen. This process requires special proteins, such as fibronectin, which normal cells also use to bind to collagen. Different tumor cells use different proteins, Liotta reports. Sarcomas, melanomas and carcinomas, which are all cancers that spread aggressively through the body, bind with laminin, a protein similar to fibronectin. Inactivating laminin with antibody can reduce the spread of these tumors in an experimental animal.

After binding to collagen, metastatic tumor cells degrade adjacent connective tissue. "Tumor cells with the highest metastatic capability have the greatest ability to degrade basement membrane [type IV] collagen," Liotta says. Liotta has partially purified an enzyme that degrades type IV collagen, and he finds the enzyme is also produced by normal cells for connective tissue turnover.

Tumor cell invasion also involves activating reactions that degrade laminin and fibronectin in the connective tissue barrier. The final step of invasion is movement of tumor cells into the zone of degraded connective tissue; a variety of attractants may be involved.

A new assay has made it possible to examine more directly the invasion of a living connective tissue barrier. Investigators had previously observed movement of tumor cells through artificial filters. Now, Liotta has substituted a barrier of natural human connective tissue—a piece of amnion, the thin membrane that encloses a fetus. Normal cells do not cross the amnion; a mother's cells stay on the outside and a fetus's cells stay within. But in laboratory experiments some tumor cells set on top of the amnion segment travel through it and can be collected on a filter below. Liotta has found, in agreement with others, that only a small proportion of a tumor's cells have the ability to cross the barrier, and thus to initiate tumors elsewhere. These cells must bind to connective tissue and apply enzymes in order to penetrate. Liotta finds that inhibitors of these enzymes block the passage of tumor cells through the amnion. He concludes, "Understanding these biochemical events of tumor invasion may provide strategies for therapeutic approaches."

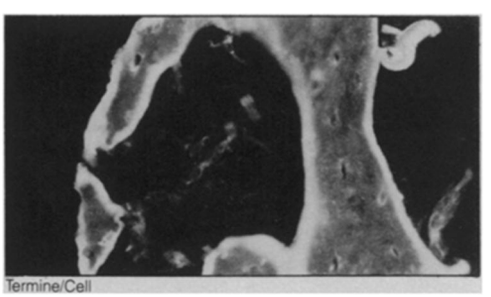
—Julie Ann Miller



Art and Photos: Liotta

To cross an experimental barrier of amnion, cancer cells attach and break down the connective tissue, creating a passage to the filter below.,

Right: Fluorescent dye reveals osteonectin in mineralizing areas of fetal calf bones. Osteonectin is thought to bind to collagen fibrils before bone mineralization.



Termine/Cell

altering how the intervening sequences are spliced from the messenger RNA of one of the two alpha-1 genes. Recent analyses of collagen genes reveal they have as many as 50 intervening, non-coding regions. In the alpha-1 gene, only about 5,000 of 40,000 nucleotides code for the polypeptide chain.

Another, even rarer, connective tissue disease is Ehlers-Danlos syndrome. It is characterized by exceptionally stretchable skin and loose jointedness. Prockop, working with Beat Steinman and George R. Martin of the National Institute for Dental Research, has found that in one case of the disease, the mutation falls near one end of the gene for the alpha-2 polypeptide chain. Consequently, the extra stretch of amino acids at that end is never trimmed off.

Different parts of the same collagen molecule thus appear to determine crucial characteristics of various types of connective tissues. If one end of the collagen molecule is altered, there is severe bone disease. If the other end is altered, the bones are normal but the patient's ligaments are affected. Absence of alpha-2 subunits—making all three type I collagen subunits alpha-1 — results in a moderate bone disease, but normal skin.

Collagen, although the most abundant, is not the only protein important in connective tissue structure. A set of proteins have been found to bind collagens with cells in the vast sea of extracellular material. These attachment factors bind to a specific sequence on a collagen molecule and also to specific receptors on a cell's surface. The factor called fibronectin, for example, is observed on the surface of cells of fibrous connective tissue, skin and embryonic muscle. In contrast, cartilage cells secrete the attachment factor called chondronectin. Some skin and body-cavity lining cells attach preferentially to collagen of basement membrane, structures that separate layers of cells. This link uses a protein called laminin. Hynda K. Kleinman of the National Institute for Dental Research suggests the genetic material for these similar attachment proteins constitute a family of genes.

Kleinman proposes a variety of potential medical uses for these attachment factors. Collagen is already added in surgery to promote wound healing. Perhaps this collagen would be more effective if the appropriate attachment factors were added too, Kleinman suggests. In other surgery implanting artificial organs, she proposes that fibronectin or similar proteins could speed up the natural process of coating implants with cells. Similarly, in cases of periodontal disease, attachment factors coating teeth might promote gum reattachment. In trauma cases — after a serious burn, for instance — fibronectin can increase clearance of dead tissue. Finally, fibronectin levels in blood might be used to diagnose prostatic cancer, and laminin levels might be used to monitor Chagas disease.

Prockop

Patients with Ehlers-Danlos (also called "rubber man") syndrome have exceptionally stretchable skin and loose joints.

A mystery about bone led John D. Termine, also of NIDR, to a different connective tissue protein. "Bone is mineralized connective tissue. Calcium and phosphate are deposited under careful physiological control," he explains. But why does the collagen mineralize in bone, but not in skin, tendon or other tissues with type I collagen? "There didn't seem to be anything special about the collagen in bone, so we turned to isolate non-collagen protein," Termine says.

Biochemical characterization of fetal calf bone produced just one protein that specifically binds both collagen and the bone minerals. Termine and colleagues call the protein "osteonectin," because it is only found in bone (and at a much lower level in the tooth tissue dentin). Osteonectin can cause binding of calcium and phosphate to collagen from other tissues, such as rat skin or tail. Termine has now purified human bone osteonectin and is making monoclonal antibody to it for use in studying bone diseases. For example, an osteonectin deficiency may be involved in osteoporosis, a bone demineralization disease affecting millions of elderly people.

Not all connective tissue disorders come from insufficient collagen or attachment factors. Important clinical problems can arise from fibrosis, when too much connective tissue becomes a fibrous scar. Under normal conditions, damaged tissue is removed after an injury and collagen participates in building new, functional tissue. But when the damage is extensive or too often repeated, normal repair mechanisms cannot cope. Instead, there is excessive, disorderly production of new tissue containing twisted, whorled and fragmented collagen fibers. This process impairs normal organ function, for example in liver cirrhosis, lung fibriotic disease and the scarring following severe skin burns.

Stephen I. Rennard of the National Heart, Lung and Blood Institute studies idiopathic pulmonary fibrosis, a chronic progressive disease of middle age, in which extensive lung scarring disrupts gas exchange between air in the lungs and the circulating blood. The key to both normal repair and fibrosis are the scavenger cells called macrophage, Rennard proposes. These cells release a factor that attracts other cells, neutrophils, that provide an enzyme to destroy collagen fibers.

Macrophage work in an additional way; they stimulate new collagen production by releasing fibronectin. The protein attracts fibroblast cells to the site of tissue injury and then serves as an attachment factor. Macrophage also release a growth factor that causes fibroblasts to divide and become more numerous. "Since fibroblasts make collagen, the end result of these processes is an accumulation of collagenous connective tissue replacing the distorted or destroyed normal lung tissue," Rennard explains.

In normal subjects, the macrophage are triggered to stimulate repair processes by a signal from the immune system. Rennard finds that in many patients with idiopathic pulmonary fibrosis, the macrophage act as if they had been stimulated even in the absence of the signal. They release the factor that attracts neutrophils and secrete high levels of fibronectin and fibroblast growth factor. Rennard thus finds the fibroblasts not guilty of causing abnormal scar formation. In a new approach to the common chronic disorders involving fibrosis, attention ought to be directed at macrophages and the stimulatory signals, he says.

All the recent information on molecular, cellular and functional properties of connective tissue are leading to a more organized concept of tissue order and disorder, says the AAAS symposium's organizer, George R. Martin. The underlying mechanisms determined for rare inherited diseases may help explain more prevalent problems. "Now we can move over into other areas of more common diseases," Martin says. □