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## Protein Provides "Tartar Control" for Joints

Researchers have identified a protein that acts as a natural form of tartar control for the joints. Mutations in the gene that produces the protein may cause a variety of arthritic diseases in vertebrates by allowing the inappropriate buildup of minerals in the joints.

The protein appears to govern the flow of mineral-reducing pyrophosphate into joint tissues. Pyrophosphate is the substance used in toothpaste to control calciferous plaque on tooth surfaces. The researchers believe that their findings could offer powerful new insights into the basic mechanisms that underlie some forms of arthritis, a group of diseases that afflicts half of people 65 and older and accounts for \$100 billion in medical costs and lost productivity each year.

Howard Hughes Medical Institute investigator David M. Kingsley and colleagues at Stanford University School of Medicine reported the discovery of the gene, *ank*, in the July 14, 2000, issue of the journal *Science*. "It had been known for twenty years that mutations in a mouse gene called *progressive ankylosis (ank)* caused severe progressive arthritis in mice," said Kingsley. "This arthritis is characterized by mineral deposition, bony outgrowths, inflammation and joint destruction. Since then, many papers have been published describing the trait, but no work had been done to find the mutation and identify the gene that controlled the disease."

Kingsley noted that although the arthritis found in the mice does share many features of human arthritis, it does not perfectly mimic any specific form of human arthritis. "The mouse ankylosis phenotype is quite severe and ultimately affects almost every joint in the body," he said. "In contrast, most forms of human arthritis are more regionalized, affecting particular joints." Although the joint disease in the mice is more severe and widespread than that seen in humans, many of the pathological processes involved resemble those seen in human arthritis. "By studying a severe form of disease in an animal model, we hoped we might be able to identify basic molecular mechanisms that may apply to milder forms of disease as well," said Kingsley.

To pinpoint the location of the gene, Kingsley and colleagues Andrew Ho and Michelle Johnson, carried out extensive cross-breeding experiments that mapped the gene to a small region of mouse chromosome 15. They

confirmed that the target gene lay within this region by inserting fragments of the chromosome segment into mice that had the *progressive ankylosis* mutation and showing that the inserted DNA segment corrected the ankylosis defect. DNA sequencing of this chromosome fragment led to the identification of several genes in the corresponding region. After comparing the sequences of normal versions of these genes with those found in the affected mice, they identified a single point mutation in one of the genes that was seen only in mice with progressive ankylosis. This mutation produced an obvious and disastrous effect on the gene product, causing a truncated protein.

"The normal product of the *ank* gene turned out to be a completely novel protein," said Kingsley. "Interestingly, it also appears to be specific to vertebrates. The *ank* gene is extremely highly conserved in vertebrates, including fish, chicks, rats and humans. However, we didn't find any homologs in any invertebrates whose genomes have been sequenced. This suggests that the gene may control some process specific to vertebrates, like the function or maintenance of cartilage and bone."

Further studies revealed that the *ank* gene is expressed during early development in regions that ultimately become the articular cartilage of joints, and in additional tissues in adult mice. "That expression pattern is particularly interesting because articular cartilage is key for normal joint function, and is normally one of the only regions in the skeleton in which minerals are not deposited. Articular cartilage normally provides a smooth, glistening, lubricated surface for joint motion," said Kingsley. In contrast, in *ank* -mutant mice, abnormal mineral deposits occur in the articular cartilage and lubricating fluid, ultimately leading to loss of mobility, inflammation, and joint destruction.

Further cell culture studies—as well as data on human families and other mice with genetically abnormal mineralization—revealed that the *ank* gene codes for a cell surface protein, ANK, involved in transporting pyrophosphate out of cells and into joint fluid, where it acts to prevent calcium buildup.

"Our functional studies suggest that the ANK protein either transports or regulates a transporter of pyrophosphate," concluded Kingsley.

"Pyrophosphate has long been known to regulate calcification, and is, in fact, added to toothpastes to prevent tartar buildup." Kingsley speculates that this gene may normally help ensure that mineral deposition is blocked in articular cartilage, and perhaps in some soft tissues as well. The human version of the gene maps to a chromosome region that has already been implicated in joint disease and mineral deposition diseases in several human families. Such findings, said Kingsley, suggest that further studies of the *ank* gene in both animals and humans could offer important new insights into arthritic diseases as well as disorders of soft tissue calcification outside of joints.