Century-long protein hunt ends with chance discovery on bone biology

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(Medical Xpress) -- In 1883, Swedish chemist Olof Hammarsten discovered that milk proteins called caseins contain not just the known building blocks of proteins, but also the chemical phosphate. It was the first hint that phosphates – which are now considered critical regulators of protein function -- are tacked onto proteins. Today, scientists know that enzymes called kinases can control protein function by attaching phosphates to proteins produced inside cells. Hundreds of kinases have been discovered and characterized, but the kinase that phosphorylates casein was never pinned down—until now.

Howard Hughes Medical Institute scientists have identified and isolated that elusive enzyme. In a study published online May 10, 2012, in *Science Express*, they report that they have uncovered the enzyme that transfers phosphate not only to milk proteins like casein, but also to proteins found in bones and teeth enamel.

“We solved this scientific puzzle that dates back to the 19th century,” says Howard Hughes Medical Institute vice president and chief scientific officer Jack E. Dixon, lead researcher of the study. “We also ended up stumbling onto this connection between this kinase and biomineralization.”

Dixon, whose lab is based at the University of California, San Diego, studies kinases, the enzymes that add phosphates to proteins, and phophatases, which remove phosphates. While most kinases are located within the cell, in the 1980s researchers started reporting that they’d

detected kinase activity (i.e. phosphorylated proteins) outside of cells. In 2008, Kenneth Irvine, an HHMI investigator at Rutgers, was studying a protein called four-jointed, which is important in the development of fruit flies. Irvine demonstrated that four-jointed had kinase activity, even though the protein was unlike any other traditional kinase. Moreover, the protein localized to the Golgi apparatus, a compartment within the cell where proteins are sorted and packaged.

“It was not clear to us that any of the approximately 580 traditional kinases had signal sequences which would allow them to end up in the same cellular compartment as casein, which was known to be secreted,” says Dixon. So, Dixon, in collaboration with Nick Grishin, an HHMI investigator at the University of Texas Southwestern Medical Center, searched databases of protein sequences to find human proteins that resembled four-jointed. They identified a family of proteins -- so little studied that its only name was Fam20 (for family 20) -- with sequence similarity to four-jointed, and found that the Fam20 A, B, and C proteins had amino acid sequences that would direct them to same part of the Golgi apparatus as the casein protein. Fam20C phosphorylated casein and many peptides on a specific amino acid sequence motif.

But discovering that Fam20C is the kinase that acts on casein wasn’t the end of the story. As Dixon’s group looked through the scientific literature, they found that mutations in Fam20C had previously been found in patients with Raine syndrome, a rare and fatal disease in which developing bones become too dense. To understand this link between Fam20C and Raine syndrome, Dixon developed cell lines that produce Fam20C containing mutations that affect the same amino acids that had been implicated in the disease.

“It turns out that every single one of these mutations inactivated the kinase and prevented it from being secreted,” says Dixon. And the targeted amino acid sequence that the researchers had nailed down

earlier wasn't just found in casein—it was found in dental matrix protein, osteopontin, and bone sialophosphoprotein, among others. All these proteins are involved in enamel and bone formation.

"In the end, what we've discovered isn't just the casein kinase," Dixon says. "It's a whole new branch on the kinase tree, a branch that seems to play very important roles in bone and teeth formation."

Phosphates are often used by proteins to bind calcium, a key ingredient of bones, teeth, and milk. So Dixon thinks the phosphorylation by Fam20C generates a calcium-binding site that is critical in the formation of bone and teeth. When the kinase is mutated, the bones or teeth don't develop correctly.

Next on the scientists' to-do list is uncovering the functions of the other Fam20 proteins—Fam20A and Fam20B, as well as working out the exact mechanism by which Fam20C works.

"This is the end of one scientific mystery," says Dixon, "but it also opens up a whole new area of kinase biology for us to explore."

More information: "Secreted Kinase Phosphorylates Extracellular Proteins that Regulate Biomineralization," by V.S. Tagliabracci et al., *Science Express* (2012).

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