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Muscle-Repair Defect Underlies Two Muscular Dystrophies

A protein defective in two types of muscular dystrophy also appears to be important in repairing damaged muscle, according to Howard Hughes Medical Institute researchers at the University of Iowa College of Medicine.

The discovery reveals the first known component of the machinery that repairs the damaged membrane in a muscle fiber. Further studies of this and related proteins could lead to a better understanding of disorders that affect cardiac and skeletal muscles.

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Howard Hughes Medical Institute investigator Kevin Campbell and Dimple Bansal led the research group that published its findings in the May 8, 2003, issue of the journal *Nature*. Campbell and his colleagues reported that their studies in mice showed that a mutant form of the muscle protein dysferlin prevents normal muscle repair in limb-girdle muscular dystrophy type 2B (LGMD2B) and Miyoshi Myopathy (MM). Campbell and his colleagues at the University of Iowa College of Medicine collaborated with Paul McNeil and his laboratory at The Medical College of Georgia.

The two forms of muscular dystrophy, which are relatively rare, have a later onset than other types of muscular dystrophy. Another research group had shown that mutations affecting dysferlin caused the muscular dystrophies, said Campbell, but little was known about how its absence caused disease.

"The only thing that was known was that dysferlin resembled a protein found in the roundworm *C. elegans* that was responsible for mediating the fusion of vesicles to the plasma membrane," said Campbell. "But nothing else was known of its function in muscle." Vesicles are the tiny cargo-carrying sacs responsible for transporting proteins and other molecules to their destinations inside cells.

“We were especially intrigued by this particular disorder because it seemed to be different than the other dystrophies we had been studying,” he said.

To explore the role of dysferlin in muscles, the researchers engineered knockout mice that lacked the dysferlin gene. Their analyses of the muscles of the knockout mice confirmed that they lacked dysferlin that was normally localized to vesicle structures and plasma membrane surrounding muscle fibers.

Significantly, the researchers found that as the mice aged, they developed the same kind of muscle pathology found in humans with LGMD2B and MM.

However, the researchers' studies indicated that the knockout mice were otherwise normal in terms of the muscle proteins known as the dystrophin-glycoprotein complex (DGC), which provides the structural support to the plasma membrane of the muscle fibers. This complex is defective in other forms of muscular dystrophy.

Electron microscopy studies of the dysferlin-knockout mice revealed that the plasma membrane surrounding the muscle fibers showed areas of disruption associated with accumulations of vesicles.

“These findings strengthened our interest in the possibility that these mice had a defect in membrane repair,” said Campbell. “So, we isolated muscle fibers from wild-type mice and damaged them to see what would happen. We found membrane patches enriched in dysferlin in the fibers that had been damaged and resealed.” This strongly suggested a direct role of dysferlin in the muscle-membrane repair process, said Campbell.

To demonstrate this role, the researchers used a dye marker to study the effects of precisely damaging the muscles of dysferlin knockout mice with a laser beam. These studies showed that the knockout mice did not have the ability to reseal damaged muscle plasma membranes. According to Campbell, the finding of dysferlin's role constitutes the first identified component of the critical membrane-repair machinery in skeletal muscle.

The discovery could yield clues to future therapies for LGMD2B and MM, Campbell said. “One possibility would be to add the dysferlin protein from the outside,” he said. “While most people wouldn't believe that the protein could pass through the membrane, a functional fragment of the protein might make it through a membrane that is already damaged.” Also, he said, therapies might be feasible that would increase the activity of dysferlin in mild cases of various forms of muscular dystrophy.

More broadly, said Campbell, the discovery opens a pathway to understanding of other muscular dystrophies, and in fact other muscle diseases. “While the study of a disease led us to this identification of dysferlin's role, we also view this as an important discovery about a basic physiological process,” he said. “As we try to isolate dysferlin-containing vesicles and characterize the proteins involved, we're hoping to discover

other genes involved in muscular dystrophies,” he said.

Campbell's team is also interested in exploring the role of dysferlin in heart and brain. Further studies may reveal the protein's role in those tissues, and whether defective forms might underlie disease.