Cell 'anchors' required to prevent muscular dystrophy

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A protein that was first identified for playing a key role in regulating normal heart rhythms also appears to be significant in helping muscle cells survive the forces of muscle contraction. The clue was a laboratory mouse that seemed to have a form of muscular dystrophy.

A group of proteins called ankyrins, or anchor proteins, were first discovered in human red blood cells by Vann Bennett, M.D. a Howard Hughes Medical Institute investigator and James B. Duke Professor of Cell Biology, Biochemistry, and Neurobiology. Ankyrins are a family of proteins that assist in attaching other proteins to the fragile cell membrane, and in the case of red blood cells, this helps cells resist shearing forces when blood is pumped vigorously throughout the body.

Bennett's team was exploring the function of anchor protein ankyrin-B (ankB) by knocking out gene expression of the gene that makes the protein. They found newborn mice missing ankB had splayed shoulder bones, which stuck out of the animals' backs like wings, rather than lying flat, a symptom of a muscular problem.

"I went back to my pediatric textbook and saw images of people with a form of muscular dystrophy who had splayed shoulder bones," said Bennett, "This opened our eyes to the possibility that, in addition to defects in controlling heart rhythm that we have studied before, the mice might also suffer from muscular dystrophy."

The team turned its attention to ankB with regard to muscle cell

organization. They knew that people with Duchenne muscular dystrophy were missing the protein dystrophin, and that dystrophin is needed for a protein complex to form and protect the cells' thin plasma-membrane layer from forces exerted by muscle cells contracting.

"Without dystrophin, you lose the entire protective complex, but nobody knew why," Bennett said. "We have found the outlines of a pathway through which dystrophin assembled this complex. The missing piece of the puzzle was the ankyrin proteins." The work appears in *Cell* journal.

The protective layer is located at a very particular place on the muscle cell membrane, where costameres, riblike structures, hold the bundled muscle cells together. This is similar to a steel cables attaching to a specific point along a suspension bridge to distribute the forces and keep the flexible bridge intact, Bennett said.

When the protective protein layer isn't present, muscle contraction forces may break the cell membrane, toxins pour in and vital enzymes stream out. The muscle cells die.

The first experiment for the new study asked if the protein dystrophin was found on the cell plasma membrane in the study animals which lacked ankB. It was not.

Beta-dystroglycan, the core component of the dystrophin-glycoprotein complex that is responsible for attaching dystrophin to the muscle membrane, also was missing, which suggested that a loss of ankyrin-B is linked to a loss of at least two key proteins in the cell membrane, Bennett said.

The researchers needed to continue their studies in adult mice with fully formed muscle cells to observe them in action, because muscle cells in culture don't have properly functioning costameres. They knew,

however, that knocking out ankyrin-B causes the mice to die soon after birth.

Fortunately, Gai Ayalon, Ph.D., a postdoctoral fellow in the Bennett laboratory, devised a method that let researchers manipulate gene expression in a specific section of adult muscle, rather than in the whole animal. "This development let us look right away at what happened in adult mice when we produced ankyrin loss only in leg muscle," Ayalon said.

Next, they studied what happened when they turned off ankyrin-G (ankG), a different anchor protein, in muscle cells. They found that the cells needed ankG to help dystrophin and beta-dystroglycan stay in place at the costameres.

Ayalon exercised the mice to learn how the muscle cells fared without ankG. The cells tore apart.

The researchers also discovered that ankB stabilized a set of structures found in all cells, called microtubules. These structures are like tracks for the molecular motors that carry the dystrophin molecules from the site where they are made to their specific destination. Ankyrin B helps microtubules align so dystrophin molecules can travel to the membrane and then ankyrin G holds them in place, Bennett explained.

"I'm excited because ankyrin-B's ability to anchor microtubules could have broad implications in many cell types," Bennett said.

Source: Duke University Medical Center