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RESEARCH NEWS

Unfurling the role of dysferlin's C2A domain

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***JGP* study (Lukyanenko et al. https://doi.org/10.1085/jgp.202513844) shows that, in the absence of full-length dysferlin, its C2A domain alone can support normal Ca^{2+} signaling and sarcolemma repair, suggesting that it could be used in gene replacement therapies.**

Mutations in the dysferlin gene are associated with several skeletal muscle diseases, collectively known as limb girdle muscular dystrophy R2. The dysferlin protein is a ~230-kDa integral membrane protein that localizes to triad junctions, the sites where transverse tubule membranes become closely apposed to the sarcoplasmic reticulum to facilitate voltage-induced Ca^{2+} release. Dysferlin is proposed to stabilize Ca^{2+} signaling at these sites as well as promote repair of the sarcolemma after injury. In this issue of *JGP*, Lukyanenko et al. report that dysferlin's N-terminal C2A domain is sufficient to support both of these activities in dysferlin-null muscle fibers, potentially simplifying the development of gene therapies for dysferlin-related myopathies (1).

For many years, researchers focused on dysferlin's role in membrane repair, but, although the protein helps repair the sarcolemma after puncture wounding or laser injury, mice lacking dysferlin can efficiently repair tears in the muscle membrane induced by vigorous eccentric contraction (2). Dysferlin-associated myopathies may arise, instead, from defects in Ca^{2+} signaling, particular after osmotic shock injury (a model for the sort of mild injury induced by eccentric contractions) (3). In the absence of dysferlin, Ca^{2+} leak from the sarcoplasmic reticulum is increased after injury, which reduces the amplitude of Ca^{2+} transients and generates Ca^{2+} waves (4).

Dysferlin contains multiple Ca^{2+}-binding C2 domains that could help stabilize voltage-induced Ca^{2+} release at triad junctions. Robert Bloch and colleagues at the University of Maryland School of Medicine previously found that the most N-terminal of these domains, C2A, was required for normal Ca^{2+} signaling (5, 6). "The role of dysferlin's C2A domain is largely to grab onto excessive Ca^{2+} and keep its levels low in the triad junctions," Bloch says.

Bloch and colleagues, including co-first authors Valeriy Lukyanenko and Joaquin Muriel, set out to investigate the specificity of the C2A domain's role in Ca^{2+} signaling (1). Transfecting full-length, wild-type dysferlin into dysferlin-null muscle fibers restores normal Ca^{2+} signaling. But versions of dysferlin with pathogenic point mutations in the C2A domain, or that lacked the domain entirely, were unable to rescue Ca^{2+} dynamics. C2A's role is specific: constructs in which the domain was replaced with homologous C2 domains from other proteins such as PKCα also failed to rescue Ca^{2+} signaling in dysferlin-null muscle fibers. A likely explanation for this specificity is that the C2A domain of dysferlin can bind Ca^{2+} more rapidly than other C2 domains can.

To investigate whether the dysferlin C2A domain is sufficient to support Ca^{2+} signaling on its own, Lukyanenko et al. tagged the isolated domain with Venus fluorescent protein and transfected it into dysferlin-deficient fibers. "It was a long shot, but it worked!" Bloch says. "A little bit gets to triad junctions and partially restores normal Ca^{2+} release."

Notably, the C2 domain of PKCα localized very efficiently to triad junctions but was unable to rescue Ca^{2+} signaling. "We decided to piggyback the C2A domain of dysferlin onto the C2 domain of PKCα," Bloch says. "Sure enough, that construct gets to triad junctions better than the C2A domain alone and is more efficient at restoring the Ca^{2+} transient and suppressing Ca^{2+} waves after osmotic shock injury."

(Clockwise from top left) Valeriy Lukyanenko, Joaquin Muriel, Robert Bloch, and Noah Weisleder.

Indeed, a construct combining the dysferlin C2A domain with two PKCα C2 domains was just as good as full-length dysferlin at rescuing the Ca^{2+} dynamics of dysferlin-null muscle fibers. Moreover, in collaboration with Noah Weisleder's group (now at the University of Kentucky), Lukyanenko et al. found that dysferlin C2A and PKCα C2 domains—either alone or in combination—are also sufficient to support membrane repair after laser wounding.

The coding region of full-length dysferlin is too large to package into adeno-associated

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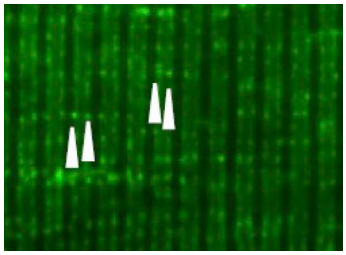

Lukyanenko et al. show that a chimeric protein containing two PKCαC2 domains and the C2A domain of dysferlin localizes to triad junctions (white arrowheads) in dysferlin-deficient myofibers. The C2A domain supports both normal Ca^{2+} signaling and membrane repair after injury, suggesting an alternative gene therapy approach for dysferlin-associated myopathies.

virus, limiting the prospect of developing gene therapies to treat dysferlinopathies. But the fact that a fusion of dysferlin's C2A domain with two PKCα C2 domains can support the two best known functions of wild-type dysferlin could greatly improve these prospects. "We are confident that this could be an alternative approach to gene therapy for dysferlinopathies and maybe other skeletal muscle diseases where control of Ca^{2+} release is compromised at triad junctions," Bloch says.

References

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