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Comprehensive Identification of Novel Ca^{2+}-Signal Transduction Pathways by Interactome Analysis

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Summary

Calmodulin (CaM) is one of the most highly conserved proteins in eukaryotic cells. It is composed of four EF-hand Ca^{2+}-binding motifs. CaM shows Ca^{2+}-dependent interaction with target proteins and consequent alteration in the biochemical functions of those proteins, resulting in the regulation of a large number of physiological responses including muscle contraction, gene expression, immune response, secretion and regulation of the central nervous system mediated by an increased intracellular Ca^{2+} concentration. Extensive studies for over 40 years have identified intracellular target proteins for CaM, including Ca^{2+}/CaM-dependent protein kinases; phosphatase; adenylate cyclase; cyclic 3', 5'-nucleotide phosphodiesterase; nitric oxide synthase; membrane receptors such as NMDA receptor and IP_3 receptor; scaffold proteins such as A kinase-anchoring protein AKAP79 and Gravin (AKAP250) and cytoskeletal proteins including unconventional myosin and actin-binding protein. Presently, CaM binding proteins continue to be discovered, suggesting that the physiological functions of CaM-mediated intracellular Ca^{2+} signaling pathways remain to be investigated. Previous studies using a proteomic approach have successfully identified CaM binding proteins from the mouse and rat brain including novel CaM targets, indicating the existence of novel Ca^{2+}/CaM-dependent signaling pathways. To search for novel target(s) of the Ca^{2+}-signaling transducer, calmodulin (CaM), we performed a newly developed genome-wide CaM interaction screening of 19,676 GST-fused proteins expressed in human. We identified striated muscle activator of Rho signaling (STARS) as a novel CaM target and characterized its CaM binding ability and found that the Ca^{2+}/CaM complex interacted stoichiometrically with the N-terminal region (Ala13–Gln35) of STARS *in vitro* as well as in living cells. Mutagenesis studies identified Ile20 and Trp33 as the essential hydrophobic residues in CaM anchoring. Furthermore, the CaM binding deficient mutant (Ile20Ala, Trp33Ala) of STARS further enhanced its stimulatory effect on SRF-dependent transcriptional activation. These results suggest a connection between Ca^{2+}-signaling via excitation-contraction coupling and the regulation of STARS-mediated gene expression in muscles.