IDENTIFYING A NOVEL VARIANT OF THE ENDOCYTIC PROTEIN CALM AND ELUCIDATING ITS IMPACT ON CLATHRIN-MEDIATED ENDOCYTOSIS

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Endocytosis is a ubiquitous process that is integral for both cellular signaling and the transportation of biomolecules inside cells. Clathrin-mediated endocytosis (CME), one of the most well understood endocytic pathways, is responsible for the internalization of receptors from the plasma membrane as well as nutrient uptake from the extracellular space. Internalization occurs through the formation of clathrin-coated pits (CCPs), sites of membrane invagination surrounded by lattices of clathrin triskelia recruited to the membrane by the adaptor protein complex 2 (AP2) and other accessory proteins1. Growth and maturation of CCPs followed by scission from the plasma membrane by dynamin results in the formation of a clathrin-coated vesicle (CCV), a process that is orchestrated by the recruitment of cargoes and additional adaptors1. One such adaptor, the clathrin assembly lymphoid myeloid leukemia protein (CALM), is thought to participate in CME as a driver of membrane curvature during CCP formation2. CALM’s ability to contribute to CME in such ways is likely due to its structure: the AP180 N-terminal homology (ANTH) domain of CALM renders it capable of binding to phosphoinositides within the plasma membrane, as well as vesicle associated membrane proteins (VAMP2, VAMP3, and VAMP8) that determine the site of vesicle fusion after internalization and uncoating1,3. Furthermore, CALM’s unstructured C-terminal domain contains binding motifs for clathrin, AP2, and other adaptor proteins such as Eps151,4.

To study the dynamics of CALM during CCV assembly by live-cell imaging, we used a knock-in CRISPR-Cas9 gene editing strategy to insert EGFP at the C-terminal of the PICALM gene. Characterization of this initial fluorescent cell clone revealed ~30% substitution of EGFP at the PICALM locus, suggesting incomplete allelic substitution or alternative splicing of the mRNA transcript. Further investigation led us to discover that the low substitution was due at least in part to the expression of a novel variant of the CALM protein. This novel isoform differs from the canonical CALM sequence by insertion of an exon containing a stop codon immediately prior to the last exon, resulting in an alternative C-terminal sequence. The new C-terminal sequence is similar to that of AP180, a neuronal homolog of CALM. Additionally, while this isoform has been annotated, it has not yet been detected or characterized. Our studies suggest that this alternative exon is expressed in both skeletal muscle and the central nervous system5,6. Complete characterization of the role each CALM variant plays in CME requires the sole expression of one isoform of the protein, which we have accomplished by refining our knock-in CRISPR-Cas9 strategy. To formulate a more complete picture of CALM’s role in CME it is essential to understand the dynamic relationships between CALM and other CME-related proteins, particularly clathrin and AP2. Furthermore, CALM has been implicated in the pathophysiology of cancer as well as in neurological maladies such as Alzheimer’s disease. Since CALM expression varies across tissues, understanding the structure, function, and expression levels of each isoform of CALM in both healthy and diseased states may result in the identification of novel mechanistic information on the pathophysiology of these diseases.

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