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Review

An eccentric calpain, CAPN3/p94/calpain-3

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ABSTRACT

Calpains are Ca^{2+}-regulated proteolytic enzymes that are involved in a variety of biological phenomena. Calpains process substrates by limited proteolysis to modulate various protein functions in the cell, and are thus called “modulator proteases.” CAPN3, previously called p94 or calpain-3, has unique features that are not found in any of the other 14 human calpains, or even in other proteases.

For instance, CAPN3 undergoes extremely rapid and exhaustive autodegradation. CAPN3 is also the first (and so far, the only) intracellular enzyme found to depend on Na^{+} for its activation. CAPN3 has both proteolytic and non-proteolytic functions. It has the interesting distinction of being the only protease, other than a few virus proteases, with the ability to regain protease function after its autolytic dissociation; this occurs through a process known as intermolecular complementation (iMOC). Gene mutations causing CAPN3 defects are responsible for limb-girdle muscular dystrophy type 2A (LGMD2A).

Unusual characteristics of CAPN3 have fascinated researchers, but have also hampered conventional biochemical analysis. In this review, we describe significant findings about CAPN3 from its discovery to the present, and suggest promising avenues for future CAPN3 research.

Contents

Abbreviations: AX, alternative exon; CaM, calmodulin; CANP, Ca^{2+}-activated neutral protease (calpain); CAPN, calpain; CAPNS1, calpain small subunit 1; CBSW, calpain-type β-sandwich domain; CTBP1, C-terminal binding protein 1; CysPc, calpain-type cysteine protease core domain; DEF, digestive organ expansion factor; EM, eosinophilic myositis; EoE, eosinophilic esophagitis; iM- and eM-activation, intra- and intermolecular activation; iMOC, intermolecular complementation; iTRAQ, isobaric tag for relative and absolute quantification; IS1 and IS2, insertion sequence 1 and 2; LGMD2A, limb-girdle muscular dystrophy type 2A; *mdm*, muscular dystrophy with myositis; NS, N-terminal addition sequence; PC1 and PC2, protease core domains 1 and 2; PEF, penta-EF-hand domain; PLEIAD, platform element for inhibition of autolytic degradation of CAPN3; TMD, tibial muscular dystrophy; TTN, connectin/titin.

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1. Introduction

Proteases are enzymes that hydrolyze peptide bonds, which are otherwise highly stable under physiological conditions. Proteases are themselves proteins, and proteolyze themselves to various extents; this process is called autolysis. The calpain CAPN3 (previously called p94, nCL-1, nCANP, and calpain-3) has such strong autolytic activity that it degrades itself within minutes, leaving researchers wondering whether such a protease can have any physiological function, and how to go about studying such an ephemeral molecule.

Despite its rapid autolytic process, CAPN3 has many important functions, and its idiosyncrasies have drawn a growing number of enthusiastic researchers. We here review the history of CAPN3 research, and introduce unusual characteristics of CAPN3 from the perspective of structure–function relationships. (Although CAPN3 is involved in postmortem meat quality and tenderization in some animals [18–31], this review focuses on physiological and pathophysiological functions of CAPN3.)

2. The discovery of CAPN3

2.1. Discovery of the calpain family

In January of 1964, **calpain** (**cal**cium-dependent pa**pain**-like protease; Clan CA, family C02; EC3.4.22.17) was described for the first time by Gordon Guroff [35]. The primary structure of the calpain catalytic subunit was determined in 1984 [37], revealing a multi-domain structure consisting of a cysteine protease domain, now called a **C**alpain-type c**ys**teine **P**rotease **c**onserved (**CysPc**) domain [38–40], a **C**alpain-type **B**eta-**S**and**W**ich (**CBSW**) domain, previously called a C2-domain-like (C2L) domain, and a **P**enta **E**-**F** hand (**PEF)** [41] domain (Fig. 1). The CysPc domain is composed of two **P**rotease **C**ore subdomains (**PC1** and **PC2**). Until 1989, most calpain studies focused on calpain-1 (previously called μ-calpain, μCANP, or calpain-I) and calpain-2 (m-calpain, mCANP, or calpain-II). Thus, these calpains are called “conventional” calpains. The terms calpain-1 and calpain-2 are now reserved for the active enzymes, which are heterodimers consisting of a larger *ca.* 80-kDa catalytic subunit, either CAPN1 (previously called μCL or μ80K) or CAPN2 (mCL or m80K), and a smaller *ca.* 28-kDa regulatory subunit, CAPNS1 (30K) (Fig. 1).

The name “calpain,” first used by Takashi Murachi in 1981 [42], was proposed to be the official name of this protease family in 1990 by Koichi Suzuki [43]. Mammals have more than a dozen calpain genes (Table 1), half of which are predominantly expressed in specific tissues or organs. Calpains with the same domain structure as CAPN1 and CAPN2 are called “classical” calpains, and the rest are “non-classical” (Fig. 1). Genetics studies have revealed that individual calpains have important and widely divergent physiological