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An Analysis of the Calcium- and Salt-Dependence of Prototypical Calpains

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Summary

Calpains are typical metalloenzymes, which are originally identified as calcium-activated neutral proteases. They are key players of calcium signaling in living organisms, and thus are involved in various basic cellular functions, such as cell division, cellular movement, and cell death. On the other hand, during the course of meat aging, they contribute to meat tenderization and release of taste components including peptides and amino acids by degrading muscle proteins.

The mammalian genome encodes 14 members of calpains. Among which, calpain-7 is thought to be a prototype, as it is most widely conserved even to yeast and fungi. The yeast ortholog Rim13 contributes to the salt- and alkaline-tolerance of yeast, by being activated in response to salt and alkaline stresses, and proteolysing and thereby activating its target substrate, the transcription factor Rim101. The calcium- and salt-responsiveness of these prototypical calpains are, however, poorly characterized, mainly because only limited biochemical research tools are available.

In this study, we have identified the Rim101 cleavage site by Rim13 as a peptide bond between Lys 533 and Ser 534. By comparing the peptide sequences around the cleavage sites of Rim101 orthologs from various fungal species, we found that the P1 residues are mostly Lys or Arg, the P2 residues are exclusively Gln, and the P5 residues are Ser or Gly. On the other hand, the sequences are poorly conserved at the C-terminal side of the cleavage site. When the well-conserved P1 residue Lys 533 was mutated to acidic Asp, or the P2 residue Gln 532 to Ala, the cleavage was inhibited. In contrast, when each of the C-terminal three residues was mutated to Ala, the cleavage was unaffected. Replacing the C-terminal three or four consecutive residues were replaced with those from PGAM5, whose cleavage is able to be monitored by the cleavage-specific antibody, the cleavage was almost normal.

From this study, a physiological cleavage site by a calpain-7/Rim13 ortholog was firmly determined for the first time in any organisms. It was also shown that the identity of the P1 and P2 residues is crucial for the substrate recognition by Rim13. Methodology to detect the Rim101 proteolytic cleavage by Rim13 with high sensitivity is being constructed.