# Pry3 short or full-length protein: where, when and how?

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The cysteine-rich secretory proteins (CRISPs), antigen 5 (Ag5) and pathogenesis related protein 1 (PR-1) are the founding members of the CAP protein family. CAP proteins are found in a wide range of species, where they are implicated in a variety of functions ranging from sterol transport in yeast, immune defense in plants, to fungal pathogenicity. These proteins have in common a conserved sequence, the CAP domain. This domain possesses signature motifs containing a histidine and a glutamate that have been proposed as catalytic residues for a possible proteolytic activity. *Saccharomyces cerevisiae* carries three CAP proteins, the pathogen-related in yeast (Pry) 1, 2 and 3. Pry3 is a secreted glycosylphosphatidylinositol-anchored glycoprotein of the cell wall. A study from Bickel and Morris, published in 2006, reports that in response to mating pheromone, an alternative promotor within *PRY3* is activated, and an alternative short *PRY3* transcript (AST) is generated. This study also indicates that the overexpression of Pry3 protein inhibits mating. Additionally, unpublish data from the Schneiter's lab has shown that Pry3 is cleaved and an N-terminal part is released into the media. The release of Pry3 in the growth media as well as the mating inhibition depend on a highly conserved glutamic acid, E96. In this work, we (1) tried to visualize if *PRY3* AST produced in response to mating pheromone was translated, and (2) tested if the release of the Pry3 N-terminal fragment was due to a conserved autocatalytic protease activity. To detect if the AST was translated, Pry3 was tagged internally with HA and mCherry. We could not record the presence of a short variant of Pry3 protein either by Western blot or by microscopy after α-factor treatment. To investigate the possible protease activity of the CAP domain, we exchanged Pry3 CAP domain with wild-type and mutant CAP domains from *Conus textile*, *Fusarium oxysporum*, *Solanum lycopersicum* and *S. cerevisiae*. In these mutated CAP domains, the glutamic acid corresponding to E96 of Pry3 was exchanged by alanine. These chimeric versions of Pry3 were then tested for their ability to inhibit mating, and to release an N-terminal fragment. All of these chimeras displayed different properties than Pry3. Their capacity to inhibit mating correlated with their expression levels and localization at the cell periphery. Furthermore, the glutamic acid 96 was important for the mating inhibition and the possibly catalytic activity of the CAP domain. The release of the N-terminal protein fragment was not correlated with the conserved E96 residues nor with mating inhibition. The discovery of different sizes of N-terminal fragments suggested, however, that the cleavage could be due to an independent extracellular protease. These results suggest that the release of the Pry3 N-terminal fragment and the production of the AST could all serve to reduce Pry3 protein levels at the mating projection where the protein might inhibit a specific step during cell fusion.

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