SL 810

SPAM (Site-specific Proteolysis Assay Method)

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Proteolysis plays a critical role in various biological processes. Conventional assay for proteolysis utilizes peptide or protein substrates labeled with chromophore or radioactivity. Here, we describe a genetic assay system by which proteolysis can be detected in vivo. This system, referred to as SPAM (Site-specific Proteolysis Assay Method), utilizes a yeast transcription factor, GAL4, and reporter genes. In SPAM, the GAL4 protein is anchored to cytoplasmic membrane via being fused to an integral membrane protein, thus it can not reach to nucleus where it functions. Then, a substrate sequence is inserted between the membrane protein and the GAL4 protein. When a protease is introduced into yeast, it cleaves the substrate site and now releases the GAL4 protein form cytoplasmic membrane. The release of the transcription factor can be easily observed in yeast by the use of the assayable reporter genes.

We have shown that SPAM can be used for cloning proteases cleaving specific substrates, studying substrate specificity of a protease, and screening protease inhibitors. We will discuss the applications of SPAM and other related methods.