

Protein Splicing: discovery and application

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Protein splicing is the excision of an intervening region of a precursor protein followed by the ligation of the flanking regions. (1,2) The intervening region is called an intein and the flanking regions are exteins, similar to intron and exon in RNA. (1,2) Protein splicing produces more than one polypeptides from one gene at the posttranslational level. (1,2)

The idea of protein splicing was raised and established by a series of experiments in the 1990's during structural analysis of VMA1 gene which found to code the catalytic subunit "a" of vacuolar membrane H⁺ ATPase in *Saccharomyces cerevisiae*. (3) The deduced amino acid of the gene found similar to the catalytic subunits of vacuolar membrane H⁺ ATPase in carrot and *Neurospora sp.* (3) But the molecular weight of the predicted protein of cloned VMA1 gene found 119kda, larger than the actual purified "a" subunit. (3) Alignment study found a 454 amino acid region present in the predicted protein but was not in the fungal or plant catalytic subunit sequences. No RNA splicing was found. (3)

A study of the TFP1 gene (which was identified as identical to VMA1) in yeast investigated the mechanism behind the discrepancy between the predicted protein and the functional protein coded by the TFP1. (4,5) The TFP1 gene was found to code for a 119 kda protein, and the authors proposed that a spacer region of 50 kda was excised from the 119 kda protein leaving a 69 kda protein which was earlier found as the catalytic subunit of vacuolar membrane H⁺ ATPase.(5,6) The 69 kda protein and 50 kda proteins both were coded by TFP-1 gene and no RNA level splicing was seen. (5) Mutation in the spacer region affected the formation of the 69 kda and 50 kda protein. (5,7) Restoration of function of the spacer region produced the 69 kda and 50 kda proteins. (5) The authors proposed the production of two polypeptides from one gene as protein splicing where the splicing activity resides within the spacer domain of the precursor protein. (5,7) The splicing was found to be a posttranslational event which involved cleavage followed by peptide bond formation between the flanking regions of the precursor protein.(7) The peptide bond formation resulted in giving a full length polypeptide of 69kda. (5,7)

The application of protein splicing is wide. The spacer domain is the intein whereas the flanking regions are exteins which are ligated after excision of intein. (1,2) Inteins have been used to ligate protein by intein mediated protein ligation (IPL). Two inteins were used in plasmid vectors to cyclize peptides which previously were chemically circularized by making disulfide bonds at the N and C terminals. But intein mediated protein ligation forms a peptide bond between the two ends of the peptides which could protect them in reduced environment, inside the cell. (8) Moreover intein mediated regulation of enzyme activity has been regulating enzyme activity in the cell. (9) Also the tadpole technique which is the fusion of DNA with an affinity protein through an intein mediated technique enables the conjugated molecule or the "tadpole" to detect and count small numbers of molecules like biotin and protective antigens of *B anthraxis* with precision and sensitivity. (10) These are few among broad applications of protein splicing.

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