

Protein Splicing in Bacteriophage Clusters

Introduction:

Protein splicing is a post translational process that results in the removal of inteins from a protein strand followed by the ligation of exteins. During this process, the inteins self-catalytically remove themselves from the protein¹, so there are no exogenous enzymes needed for this process to occur.⁶ Protein splicing is also crucial to maintaining the function of the cell. Regulation of splicing patterns can be used to alter cell function as a response to environmental

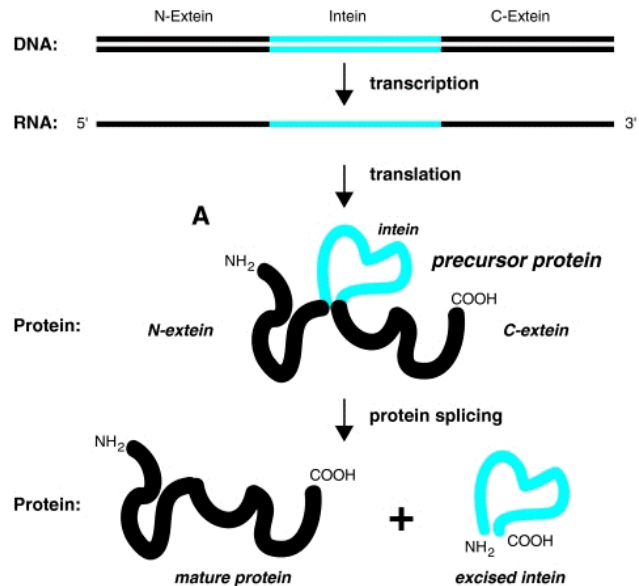


Figure 1: How protein splicing fits in with the central dogma. Protein splicing happens after translation.

conditions changing.² The actual mechanism of protein splicing takes four steps.⁴ In the first step, a nucleophile attacks the peptide bond linking the N-extein and intein forming an ester. The next step is a transesterfication in which the N-extein is shifted to the C-extein. The intein is then cleaved in the next step. The final step involves the ester bond between the exteins becoming peptide bonds in order to join them together.⁴ There are four different types of inteins including alanine inteins, mini-inteins, maxi-inteins, and trans-splicing inteins.³Intein sizes vary in length from about 134 amino acids reaching up to 650 amino acids.⁶ Protein splicing has been synthesized in a lab setting using such processes as phosphorylation, lipidation, acetylation, glycosylation, and ubiquitination.⁷ There are 550 genes registered in intein database due to characterized sequence motif, but only a few dozen actually been tested.⁴ One of these inteins is

from the mycobacteria phage, Bethlehem. Bethlehem is considered to be part of Class 3 inteins since it contains N-terminal splice junctions that do not begin with Thr, Cys, or Ser that are instead embedded in the intein (Figure 1)⁵ and instead consist of a Cys in the middle of the intein that attacks the N-terminal splice junction. It is one of the few known to date. Protein functions of genes within phages are split into phams with those that are related to that protein. The proteins in each pham should have a similar sequence and thus the same function within the phage/bacterial cell. The purpose of this experiment is to see if this is the case in splice junctions and inteins in proteins that are in the same pham as the intein sequence from Bethlehem, pham

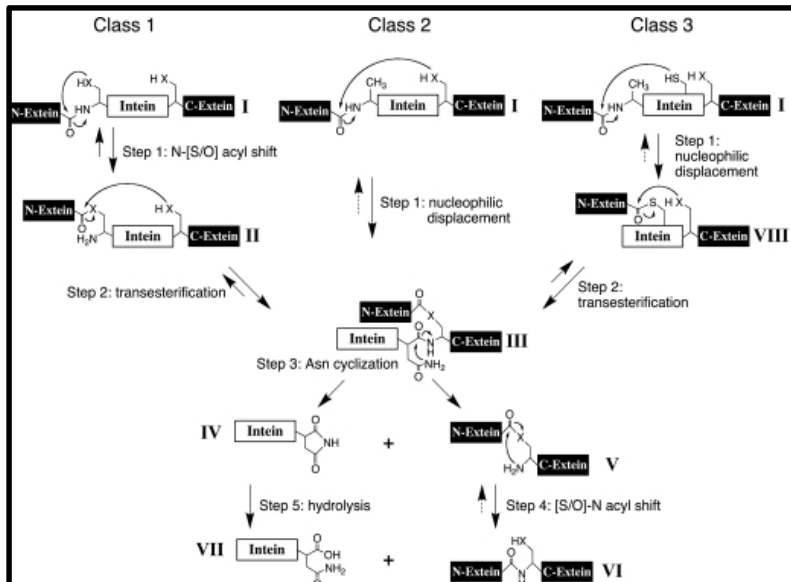


Figure 2:
 The three intein classes differ in mechanisms. Class 1 contain N-terminal Ser or Cys allowing an acyl shift initiating the splicing reaction. Class 2 contains a Cys⁺¹ located near the C-terminal that attacks the amide bond at the N-terminal splice junction. Class 3 mechanisms occur when a cis from the intein attacks the N-terminus junction.

216.

Experiment:

The aim of this experiment is to look at protein splicing inteins in pham 216 to determine if it is similar among all other phages in the pham/cluster. Tori, K. et al. ran an experiment on a phage, Bethlehem, whose intein protein is in pham 216, was ran to examine the lack of N-terminal and C-terminal inteins that characterize the third class. In

this experiment, I want to see if other mycobacterial phages within the same pham have the same sequence and possibly mechanism for protein splicing, if one at all. In order to determine that, an

experiment similar to the one run for Bethlehem will be implemented to determine if the same

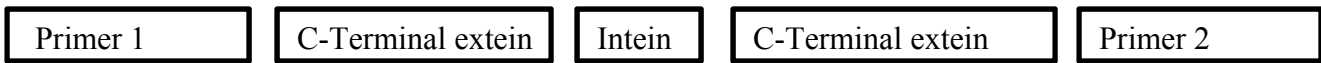


Figure 3: How the primers will cut the protein including the inteins.

motifs can be used. PCR is used in order to amplify DNA that encodes for the desired intein which is amplified from the phage's DNA (any phage in pham 216) using the primers (1)

5'-ATGTTTCTCGAGGTCTCCCAGGACCAGCCT-3' and (2)

5'AATGAAACTAGTGAACGTGTTCTTCGTGTT-3' (same used on Bethlehem)⁵. Mutations

are then introduced to the DNA of catalytically important residues in order to help isolate the precursors—intein C & N termini, C+1S, and C1S. The DNA is then overexpressed in *E. coli* using plasmids and then separated from the cells and placed in a buffer solution. The proteins are then denatured and ran through gel electrophoresis and analyzed to look at the bands of the proteins to determine if they are similar or different from the results of Bethlehem.

Discussion:

If the experiment turns out as predicted by phams that the sequences are similar and should result in the same proteins, the resulting gels should be similar in bands. Of course, there may not be any splicing that occurs since the DNA may not contain a class 3 intein since it is this class simply by the placement of 1 Cys amino acid and the mutations of the DNA will only work for a class 3 intein. It could also lead to the intein being class 3, just having a different amino acid than that of Bethlehem's—have Thr or Lys like the other two classes have in addition to the Cys. If this is successful, there will be more known class 3 inteins that could be studied further and the mechanism could possibly be implemented in the future to rid mutations from proteins.

References:

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Figures:

Figure 1:

http://4.bp.blogspot.com/_avzLfLUJNqM/TMi2UomrHiI/AAAAAAAAABYc/yqxaEk1fshk/s1600/inteins.gif

Figure 2:

<http://www.ncbi.nlm.nih.gov.proxy.library.vcu.edu/pmc/articles/PMC2807308/figure/F1/>