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BMS

Judging Dept.

Karla Lehtonen

Student

BMS

3

Marlene Belfort

Dept or Program Years in program

Mentor

Testing Intra-Molecular Interactions Involving the Linker Region of the I-TevI Endonuclease Using Split-Constructs

Author (s)

Karla Lehtonen, Qingqing Liu, Victoria Derbyshire, and Marlene Belfort

I-TevI is a GIY-YIG endonuclease encoded by the td intron of the bacteriophage T4. I-TevI initiates the first step of intron homing by making a double-strand break in its DNA target the homing site. The N-terminal catalytic domain and the C-terminal DNA-binding domain are joined by a 75-amino acid flexible linker. This linker can be divided into three regions: the DI region (deletion intolerant), the DT region (deletion tolerant) and a zinc finger. The DI region is important in the activity of the catalytic domain. The DT region and the zinc finger are required for positioning of the catalytic domain at a set distance from the primary binding site during the cleavage reaction.

We are probing for intra-molecular interactions between different parts of the protein by splitting I-TevI into two parts at selected positions in the linker region by creating a two-gene operon with the first gene coding for the catalytic domain and part of the linker region while the second gene codes for the remainder of the linker region and the DNA binding domain. We are also creating a two-gene operon where we have reversed the position of the C-terminal DNA binding domain and the N-terminal catalytic and linker domains on the coding sequence. We hypothesize that if there are intra-molecular interactions, they may be sufficient to hold the catalytic and DNA binding domains together even if they are not covalently joined. We will be testing for these intra-molecular interactions with cleavage assays.