

Genetic and Structural Studies on the Mu Gin Protein

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Site-specific recombination involves the formation of ordered nucleoprotein complexes. For the recombination process that leads to the inversion of the G-region in Mu, a synaptic complex is formed between two Gin dimers bound to the inverted repeats that flank the G-region, and Fis molecules bound to the enhancer. Until now it is not clear what kind of interactions between the different molecules of Gin take place during the subsequent steps of the recombination reaction, and whether Fis is only needed for the formation of the complex or whether it has some additional role in the strand exchange.

To get more information about the protein interactions between the different molecules of Gin we did *in vitro* cross-link experiments on the purified protein in solution. We found that in the Gin molecule the N-terminal region of 61 amino acids is in close contact with a second Gin molecule upon dimer formation. Surprisingly we found that under oxidizing conditions, a dimer of Gin molecules is also formed without the presence of a chemical crosslinker. We can show that this dimerisation under oxidizing conditions is caused by disulfide bond formation between one or two of the cysteines which are present in the wildtype protein at the positions 24 and 27.

We have also tried to isolate mutants in Gin which are disturbed in the putative interaction with Fis. For this we have isolated 25 independent Gin mutants that still normally bind to the recombination site but are affected in one or more of the subsequent steps in the reaction. In these mutants we introduced a mutation which in the wildtype Gin protein causes a Fis-independent phenotype (A. Klippel et al., EMBO J. 7 (1988) 3983-3989). This was done because we expected that mutants that were only disturbed in the interaction with Fis will function again when they are Fis-independent. Among the Gin mutants we have isolated, we found several mutants with the expected phenotype. The location of these mutations in the protein structure and their properties will be discussed.

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