Integration of the phage λ into secondary attachment sites using Integrases with altered specificity

Theses of the doctoral dissertation

Edit Szameczné Rutkai

Supervisor: Dr. Dorgai László

Bay Zoltán Foundation for Applied Research

Institute for Biotechnology

Szeged, 2006

Introduction

One of the most studied virus-bacterium system is that of the *Escherichia coli* and the phage λ. Like other temperate bacteriophages, the phage λ can follow two strategies after infecting bacterial cells. In the lytic cycle the phage lisates the bacterial cell while approximately one hundred new viral particle is formed. The other possibility is the lisogenic cycle when the phage DNA integrates into the chromosome of the host cell.

This insertion takes place using the site-specific recombinational system which following recognition of the unique sequences on the bacterial chromosome and phage DNA fuses the two genomes by a reciprocal recombination. The inserted phage is called prophage.

The key enzyme of the recombination is the Integrase that catalyzes the cleaving of the DNA strands, their swapping and rejoining. The sequences where the recombination takes place and are recognized by the Int are called *attachment* (*att*) sites.

Beyond the primary *att* sites both the bacterial chromosome and the phage contain so-called secondary *att* sites. The integrational efficiency at these sites is lower by several orders of magnitude than that

of the primary *att* sites. Integration into secondary att sites is undetectable if the primary *attB* site is present on the chromosome. The structures of the secondary sites are similar to the structure of the primary sites and their sequences share a significant homology too.

Similarly to the phage λ, the HK022 also belongs to the temperate bacteriophages. Former studies comparing the site-specific recombinational systems of two phages revealed that the mechanism of both the integration and the excision is very similar. In spite of the structural and regulational analogy, none of the two Integrases can catalyze efficiently the integration or excision of the other phage.

The mechanism of the evolutionary changes in the protein-DNA interactions can be understood by studying the differences and similarities of the HK022 Int and the λ Int. As a result of their evolution, the two proteins have different recombitional site-preference, but their sequences share a high level of homology and a low level of cross-reaction can be detected.

The question is that how can a new specificity evolve from an existing one in a way that the system would keep its functionality during the whole process, because the loss of compatibility between the Int and its recognition site would decrease the efficiency of lisogenisation.

During former studies leading to my project, the elements responsible for the different specificity in the two site-specific recombinational systems of these phages were determined. The amino acid residues responsible for the difference in the specificity of the two system and also the nucleotides in the core-type Int binding sites that blocks recombination catalyzed by the non-cognate Integrase were identified.

Based on the *in vivo* recombinational efficiency of different combinations of the wild type or mutant Integrases and the wild type or mutant *att* sites we set up a hypothesis, that describes a possible way how the specificity of an existing *att*/Int system can change during the evolution: one or more Int mutations relax the specificity of the recombinase and further mutations restrict it, resulting in that only the new one but not the original sequences will be recombined efficiently. Finally, new mutations in the Int and in the new Int-binding sites will refine the system.

Specific aims

The hypothesis describes a possible mechanism for the change of specificity, but its validity is still unknown.

It is unlikely that the specificity-change in the direction of λ → HK022 can happen under non-experimental circumstances as it was modeled experimentally, without loosing functionality even temporarily, because of the complexity of the system and the relatively high number of the expected mutations. The Integrase with relaxed specificity should find one or more sequences that can be recombined with appropriate efficiency.

For this purpose the secondary att sites of the host cell are good candidates since their sequences are similar to the primary *attB* and poorly but can be recombined by the wild-type Int.

My specific aims were to investigate if the secondary *att* sites could play a role in the change of the specificity of a recombinational system and to determine the mechanism of this possible pathway.

Results and discussion

The efficiency of the integration into the *Escherichia coli* chromosome using all of the secondary *att* sites was measured *en masse* using λ phages carrying the wild type or mutant Integrases and the ratio of the integrational efficiencies at primary and secondary *attB* sites was determined.

In Southern hybridization assay the secondary sites used most frequently by phages carrying the wild type and mutant Int was detected.

The *att* regions of prophages integrated into secondary sites were amplified in one-sided PCR using the population of thousands of single lisogen strains as a template. By determining the sequence of these PCR products, 19 new secondary *attachment* sites were identified. Having known their sequences, most of the isolated secondary att sites were identified on the picture of the Southern-hybridization.

459 single lisogen was analyzed and the secondary site where the integration took place was determined, resulting a quantitative description about the usage of these sites

The most outstanding result of the analysis of the secondary *att* sites is the strong conservation of the first three nucleotides of the

overlap region. Previous data showed that the sequences of the *overlap* regions in the recombining *att* sites should be identical, but the sequence itself is indifferent. Our experimental data proves that this identity is important only for the first three nucleotides of the *overlap* regions in the case of integrational events at the secondary sites.

By examining 197 independent insertions we demonstrated that unidirectional segregation is the rule when the right sides of the two *overlap* regions are multiply mismatched, that is that all of the *attL* overlap regions had the sequence of the λ and all of the *attR* overlap regions had the sequence of the secondary sites. This observation can be explained by the inhibition of the second strand swap because of the differences in the sequences of the right side of the *overlap* regions, so the Holliday structure created by the first strand exchange will be resolved not by the Int but some other mechanism, for example by the bacterial homologous recombination or replication.

The results presented in this work led us to set up a model called chromosomal jumping. According to the model an *attR* transducing phage is formed in which the *attR* predominantly preserves the structural complexity of attP and after having some adaptive mutation it can ensure the rapid restoration of integrative function.

We have examined two predictions deduced from our model. In the first experiment artificial plasmid substrates, in the second an *attR* transducing phage was used. In the case of the transducing phage we proved that it has the structure that was predicted by our model, and its integrational and excisional functions are unimpaired. In the case of the artificial plasmid substrates the integrational and excisional efficiencies were measured using different *att* sites.

The observed significant difference between the efficiencies of some integrational and excisional reactions in the case of certain substrate/Int combinations was unexpected. The results from these experiments are compatible with the mechanism described in the model and confirm the availability of it.

The possible role of the secondary *att* sites in the change of specificity of the site-specific recombinational system was investigated according to our initial aims. A model was set up and some elements of it were proved by experimental data. Of course, we cannot say that the examined mutations of the Int protein and the secondary sites were playing role in the change of specificity, but our results confirm its possibility.

Methods

Beyond the standard methods of microbiology and molecular biology the following methods were used in our experiments:

- the integration of the λ phage into the *E. coli* genom was measured *in vivo*, following phage infection the number of lisogen colonies was divided by the number of all the surviving colonies,
- the integrational and excisional efficiencies of plasmid substrates was determined by following the changes in the expression of the lacZ gene by determining the ratio of the blue colonies in the presence of X-gal substrate,
- the most frequently used secondary sites were identified in one-sided PCR reactions and the frequency of their usage was determined using the same method.

Publications

E. Rutkai, L. Dorgai, R. Sirot, E. Yagil and R. A. Weisberg: Characterization of secondary attachment sites used by phage λ, and a role for such sites in changing phage insertion specificity
J.Mol.Biol. 329 (5) 983 – 996, 2003

E. Rutkai, A. György, L. Dorgai and R. A. Weisberg: The Role of Secondary Attachment Sites in Changing the Specificity of Site-Specific Recombination
J. Bacteriol. 2006 (*in press*)

T. Bubán, **E. Rutkai**, L. Dorgai and Thomson, S. V.: Prediction of infection risk on the basis of weather-related factors and *Erwinia amylovora* colonization in apple and pear flowers
Int. J. Hort. Sci. 10 (2), 39-54, 2004

S. Sloan, **E. Rutkai**, R. King and R.A. Weisberg: Persistence of RNA mediated antitermination (manuscript)

Oral presentations

E. Rutkai, L. Dorgai, R. Sirot, E. Yagil and R. A. Weisberg: Conserved elements in secondary attachment sites used by λ and *int* mutants with altered insertion specificity.

Analytical Genetics, Santorini, Greece, Oct. 1-6, 2002.

E. Rutkai, S. Sloan, R. King and R. A. Weisberg: Persistence of RNA mediated antitermination.

The New Phage Biology, ASM Conferences, Key Biscayne, Florida, USA, Aug. 1-5, 2004

Posters

E. Rutkai, L. Dorgai, R. Sirot, E.Yagil, and R.A. Weisberg: Secondary attachment sites for phage λ and the evolution of phage insertion specificity

Molecular Genetics of Bacteria and Phages, Cold Spring Harbor, USA, Aug. 20-25, 2002.

Acknowledgement

I am grateful to a number of people who helped me:

- I thank Dr. László Dorgai for supervising my Ph.D. work, for his useful advices and for helping my professional advancement and carreer.
- I am especially grateful to my collegue, Andrea György, for her contribution to the experimental work.
- Many thanks are due to Aranka Bálint and Andrea Vörös for their everyday help and valuable assistance.
- I thank Dr. István Kiss and Gabriella Jankovicsné Urbán for their competent critique of the earlier version of my thesis.
- I thank Dr. Miklós Kálmán, the director of the Bay Zoltán Institute for Biotechnology for providing the facilities for my research.
- I thank to Dr. Robert Weisberg for the possibility to learn about bacteriophages and transcription.