“REGULATORY STRATEGIES FOR FORMULATION OF MACROMOLECULAR COMPLEXES INITIATING THE DNA REPLICATION PROCESS OF PHAGES CARRYING SHIGA TOXINS GENES"

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ABSTRACT

Escherichia coli bacteria are commonly found in the environment, as well as being an important component of the natural bacterial flora of the human intestine. Although they are commensals, some strains can also be pathogenic. Strains that produce Shiga toxins (STEC) are among the most dangerous pathogens. Contagions with these strains are particularly dangerous, because up to now there are no drugs that could help fight infection. Shiga toxins, the main virulence factor of these strains, are encoded by the genes present in Stx bacteriophage genome. These phages belong to the group of lambdoid bacteriophages which show a large similarity in structure to the model organism of molecular biology - λ phage. Stx bacteriophages are present in *E. coli* cells in the form of prophage, which means that their genome is integrated into the bacterial genome. Under the influence of various factors and conditions prevailing in the cell, transition of the phage into a lytic cycle is possible, which involves the excision of its genome, efficient replication of DNA, and toxin production. An effective process of amplifying the bacteriophage genome is essential for producing a dangerous Shiga toxin. The deepening of processes regulating the initiation of DNA replication can therefore contribute to a better understanding of the pathogenicity mechanisms of STEC strains.

The bacteriophage λ DNA replication has been well understood, but its regulation is still not clear. It is known that this is a very complex process involving both phage elements and protein machinery of its bacterial host. A lot of information was provided by *in vitro* studies. λ phage replication begins with the binding of the O protein to 4 DNA sequences in the origin of replication, named iterones. The key role in initiating of the process of amplification of the genetic material of phage λ is played by bacteriophage-encoded O protein and transcriptional activation of the origin of the replication. Stx bacteriophages show a slightly different structure of the DNA replication origin as well as the initiator O protein. These differences may lie at the base of altered regulation of the initiation of replication of the genetic material of Stx phages compared to the λ phage. The latest research indicate that this key process in the Stx phage development cycle can be less dependent on transcriptional activation. It has been shown that some Stx phages multiply in *E. coli* strains with reduced transcription from the p_R promoter, in contrast to the wild type phage.

In this thesis, I present a comparison of origin of replication and O proteins of λ and Stx phages. In my research, I established protocols for the purification of the Stx phages P27 and 933W O proteins. I also investigated the effect of the different construction of initiators and the origin of replication on the assembly of the O-some complex. Stx proteins, like the λO protein, exist in the form of a dimer. Only 4 iteron sequences are bound during the initiation of Stx DNA replication, despite the presence of 6 such fragments in the Stx *ori*. The function of the other two iterons is not clear. As these sequences are found in the gene coding for Stx proteins, the sequences of the proteins themselves are also extended compared to the λ phages. Therefore,

proteins O of phages P27 and 933W have 13 additional amino acids. They can act as a space barrier, thus affecting the lesser packing of the O-some Stx complex compared to the structure found in phage λ. Such structure of the DNA replication initiation complex may determine its lesser dependence on the processes occurring in the host cell, also on the transcriptional activation. Evidence for the independence of Stx O-some assembly from active transcription was provided by footprinting technique. A lack of the need to involve elements of the bacterial cell structure for efficient reproduction of the bacteriophage genome, may indicate the specialization of Stx phages.