III.P.1

Changes in kinetics of DNA synthesis of plasmids replicating in *Escherichia coli* cells devoid of the Hfq protein

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The Hfq protein belongs to the family of thermostable bacterial proteins. The occurrence of its orthologue genes has been reported in nearly half of the sequenced bacterial genomes. In bacterial cells, it is present at high abundance in the cytoplasmatic fraction associated with ribosomes, and it is located close to the bacterial membrane. Hfq is known as the major global riboregulatory factor. Many functions of this protein, associated with RNA metabolism, have been reported, including RNA chaperone activity that facilitates sRNAs base pairing with their target mRNA, and interactions with proteins involved in mRNA decay. Nevertheless, recent studies indicated that Hfq is also a DNA-binding and nucleoid-associated protein. Association of Hfq to DNA has been observed both *in vitro* and *in vivo*. Furthermore, our previous studies revealed that *hfq* gene mutation affects plasmid DNA replication.
This study was focused on the replication of ColE1-like and pSC101 plasmids in *hfq* mutants. We have performed *in vivo* plasmid replication experiments by measuring the levels of radioactive thymidine incorporation during plasmid DNA synthesis. Total plasmid DNA content was determined using fluorescence-based quantification method. Our results revealed differences in kinetics of plasmids' replication during the bacterial growth. Our findings support the previous suggestion on the role of Hfq as the regulator of propagation of some replicons.

Key words: Hfq protein, plasmid, replication, ColE1-like plasmids, pSC101 replicon

III.P.2

DNA replication model of plasmids pIGRK and pIGMS31-a 2D agarose gel electrophoresis approach

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pIGRK and pIGMS31 are two plasmids originally isolated from a clinical strain of *Klebsiella pneumoniae*. They are small (~2.5 kb), cryptic and each of them has only two open reading frames (ORF). One of the ORF's encodes a putative replication protein. The second ORF, in case of pIGMS31, encodes a protein with similarity to the mobilization protein, engaged in the conjugation process. Protein encoded by the second ORF of thepIGRK plasmid possesses similarities to phage integrases. Despite their cryptic nature, these plasmids can transfer to other bacteria, sometimes even only distantly related. This suggests that these plasmids may play an important role in the horizontal gene transfer or act as suicide vectors.
pIGRK and pIGMS31 belong to the pHW126 plasmid family, and thus are suspected to replicate by the rolling circle model. However, studies by 2D agarose gel electrophoresis propose that replication of the mentioned plasmids is more complex and may involve the theta model.
To study replication intermediates of pIGRK and pIGMS31 replicons, plasmid DNA was linearized by restriction digestion. This was followed by 2D electrophoresis where the DNA molecules are first segregated mostly according to mass. When migrating in the second dimension, the molecule's electrophoretic mobility is greatly affected by its shape. Emerging patterns are visualized by Southern blot and further analyzed. The results of these experiments along with their interpretation are going to be presented.

Key words: replication, plasmid, electrophoresis

III.P.3

Initiation of chromosome replication in *Campylobacter jejuni*

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Campylobacter jejuni is one of the most common causes of gastroenteritis worldwide. Its genome was sequenced 15 years ago, yet there is still little information concerning *C. jejuni* biology including the cell cycle of this bacterium. One of the most important check-points of the cell cycle progression is initiation of chromosome replication. Chromosome replication in bacteria begins with binding of the DnaA protein to a unique DNA region termed *oriC*. The initiator protein interacts with specific DnaA-binding sites (DnaA boxes) localized in *oriC,* which subsequently leads to DNA duplex destabilization within the AT-rich region (DUE). The emerging replication bubble allows helicase loading and further assembly of the replication machinery (i.e. replisome formation).
On the basis of the *in silico* analysis, we discovered that *oriC* in *C. jejuni*, similarly to *Helicobacter pylori*, consist of two subregions *oriC1* and *oriC2*. The DnaA-dependent unwinding occurs at *oriC2* and, similarly as in *H. pylori,* DnaA binding to the DUE-proximal subregion is sufficient to trigger helix destabilization. There are at least seven DnaA boxes in *oriC*; their consensus sequence partially corresponds to the consensus *H. pylori* DnaA box. The subregions exhibit different affinities towards DnaA (*oriC1>oriC2*). The binding of DnaA to full length *oriC* seems to be cooperative because DnaA binding to *oriC* is higher than the combined binding to each of the subregions. This suggests that, similarly as in case of other bipartite origins, in *C. jeujni oriC* the DNA loop is formed upon DnaA binding to each of the subregions. The Cj0545 protein, a HobA homolog, decreases the oligomerization of the initiator protein upon origin binding but simultaneously stabilizes the initiation complex. Cj0545 apparently arranges DnaA upon interaction with *oriC* in order to form a structured nucleoprotein complex. Further work should help to characterize the structure and dynamics of the *C. jejuni* orisome formation.

Keywords: DnaA, initiation of chromosome replication, *Campylobacter*, ε proteobacteria

III.P.4

Interactions between the origin of replication and the initiator O proteins of Stx phages – influence on the DNA replication initiation process

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Effective initiation of DNA duplication of Shiga toxin-converting (Stx) phages is essential for efficient production of Shiga toxins in enterorrhagic *Escherichia coli* (EHEC) cells. The first step of the DNA replication process in lambdoid bacteriophages is binding of the O protein to phage DNA at the origin region which initiates formation of the pre-replication complex. Genomes of Stx phages reveal a similarity in structure and nucleotide sequence to that of bacteriophage λ. However, specific differences in factors involved in DNA replication initiation were demonstrated to cause remarkable changes in the regulation of this process between the related phages.
We examined the impact of differences between the Stx phages and the λ phage in the sequences of the origins of replication, as well as in the O proteins, on the control of replication of genetic material, using a variety of *in vitro* methods.
Our data showed that variants of the O protein bind in the dimeric form to two different phage origins in a similar way, with only a few alterations. This similarity was surprising since λ origin contains four nearly palindromic repeats, dubbed iterons, recognized by the initiator protein, whereas the Stx phage sequence includes six of them. This indicates that only four of Stx iterons are involved in the formation of the initiator complex, known as O-some. Differences in the complex assembly seem to result from various affinities of variants of the O protein to each iteron or alterations in their oligomerization properties.
Studies on the regulation of replication of the Stx phages may lead to better understanding of the basic control processes of the lambdoid phages' development and pathogenicity of EHEC strains, as well as facilitate the design of new methods of detection, prevention and therapy of EHEC-caused infections.

Key words: DNA replication, Stx phages, initiator O protein, λ phage, STEC

III.P.5

The effective introduction and replication of ColE1-like and P1 plasmids in marine bacterium *Shewanella baltica*

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Shewanella baltica, from the *Shewanellaceae* family of marine bacteria, was isolated from the Baltic Sea. This species has a potential for bioremediation, especially in the specific aquatic sediment and soil environments, rich in Mn and Fe oxides, where *S. baltica* could play a powerful role in the environmental cycling of these metals. For further studies, it is important to identify plasmids which can be introduced and maintained in *S. baltica* to serve as vectors to transfer and express recombinant genes. However, no information about possible use of plasmid vectors in this species has been reported till now. Moreover, the information about the ability of *Shewanella* genus representatives to accept and maintain plasmids is quite limited. In this work, we report the ability of *S. baltica* to accept and maintain plasmids with different *origin* of replication. Research methods include i) electroporation for introduction of plasmid DNA into *S. baltica*, ii) assessment of transformation efficiency in comparison to those observed in *Escherichia coli*, iii) plasmid stability evaluation to determine plasmid replication and maintenance.

We found that *S. baltica* could be transformed with plasmids from ColE1 family and by P1 bacteriophage-derived plasmid. However, the transformation efficiency of *S. baltica* was significantly lower comparing to the results obtained for *E. coli*. To employ plasmids as vectors for genetic manipulation, it is crucial to known whether they can be stably maintained in the cell. Thus we tested the maintenance of four plasmids (pZE21, ori ColE1; pZA31, ori p15A; pECFP-c1, ori pMB1; pSP102, bacteriophage P1 origin), that could be introduced into *S. baltica*. We found that all tested plasmids were lost from the bacterial population, during cultivation in the absence of antibiotics. However, these plasmids can be employed for introduction of heterologous DNA and genetic manipulation of *S. baltica* providing relevant antibiotic selection.

Key words: *Shewanella baltica*, marine bacteria, plasmid maintaining, *origin* of plasmids replication

III.P.6

Analysis of the DNA replication rate of *Escherichia coli* strains harboring mutations in genes of the central carbon metabolism pathway

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DNA replication and central carbon metabolism (CCM) are crucial components for cells sustenance and division. The correlation between them is currently being examined and the evidence exists for the link in *Bacillus subtilis* and *Escherichia coli*, but the nature of this bond is yet to be determined. Initiation of DNA replication is an essential step, which occurs at an unique site – origin (*oriC*), proceeds bidirectionally and ends at terminus (*ter*) sequences that are located on the opposite to *oriC* site of bacterial chromosome. Initiation of replication is precisely regulated. It takes place only once per origin but in cells growing in rich medium, new round of replication starts before the previous one is completed, thus creating a phenomenon called overinitiation. CCM provides the cell energy from nutrients and supplies the precursors for biosynthetic pathways. It consists of many pathways which create a functional network.

The main purpose of this work was to define the nature of the link between DNA replication and CCM. To get insight into the replication kinetics we compared *E. coli* wild type and metabolic mutants growth and replication rate. Experiments were performed by qPCR method with primers for *oriC* and *terC* sequences.

Our analysis demonstrated that there indeed is a difference in growth and replication rates between certain mutants. In some of them the initiation is triggered three times per one cell cycle (glycolysis and gluconeogenesis mutants), in other twice (TCA cycle mutants). The biggest reduction (in comparison to wild type *ori*/*ter* ratio 3.42) is visible within the mutants in overflow pathway genes (*pta* – 1.57, *ackA* – 1.92). These differences might occur because of the alteration in concentration of some metabolic intermediates and such situation may result in intracellular pH change. This may have direct or indirect influence on replication by regulating activity or transcription of replication regulators genes respectively.

III.P.7

In vitro and *in vivo* analysis of replication initiation proteins encoded by pIGRK and pIGM31 plasmids of *Klebsiella pneumoniae*

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pIGRK and pIGMS31 are members of the pHW126-like family of plasmids, whose replication proceeds *via* rolling circle mode (RCR). Comparative analysis revealed that amino acid sequences of replication initiation proteins of pIGRK and pIGMS31 (RepR and RepM, respectively) lack conserved motifs typical for catalytic domains of RCR plasmid initiator proteins. Surprisingly, both RepR and RepM proteins contain helix-turn-helix (HTH) DNA binding domains, typical for Rep initiators of theta replicating plasmids. In contrast to RepM, RepR contains an additional C-terminal coiled coil motif involved in protein dimerization. Electrophoretic mobility shift assays (EMSA) proved that the purified RepR and RepM proteins bind specifically (i) to predicted double strand replication origin (dso) of their cognate plasmids and (ii) to promoter regions of the RepM and RepM-encoding genes (P_{rk} and P_{ms}, respectively). These observations were confirmed by *in vivo* experiments. Using two-plasmid systems we showed that RepR and RepM, when delivered *in trans*, were able to initiate replication of plasmids containing putative dso of pIGRK or pIGMS31. It was also demonstrated that the Rep proteins regulate activity of the P_{rk} and P_{ms} promoters. To our best knowledge this is the first report showing the ability of Rep proteins of RCR plasmids to act as transcription regulators.

Key words: RCR, plasmid, *Klebsiella pneumoniae*

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III.P.8

Bacterial growth and nucleic acid synthesis of *Escherichia coli* is affected by metabolites from the central carbon metabolism

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The replication of DNA is a basic biological process, necessary for each living organism. Central carbon metabolism (CCM) as a center of cellular energy metabolism is crucial for all life functions of organisms, providing energy from nutrients and supplying the precursors for biosynthetic pathways. In our previous studies we employed temperature sensitive (*ts*) *Escherichia coli* mutants in genes coding for replication proteins, with their characteristic features of filamentous morphology and growth inhibition in the restrictive temperature. We observed that this defect could be suppressed by deletions of certain genes encoding enzymes of the CCM, *pta*, *ackA* (overflow pathway), and *gpmA* (glycolysis/gluconeogenesis). This led to the hypothesis that the increased levels of metabolites as a result of dysfunction of certain CCM enzymes may be responsible for the influence on the replication of DNA.

The aim of the research was to elucidate the effects of metabolites: pyruvate, acetate, succinate and fumarate on replication mutant strains: *dnaA46, dnaB8, dnaC, dnaE486*, *dnaG*, *dnaN159* and MG1655 (control). In order to study the metabolites effect on DNA synthesis, the kinetics of these processes were assessed by the incorporation of radioactively labelled [^{3}H] thymidyne in the restrictive temperature. Additionally, microscopic analysis of metabolites effects on bacterial morphology was performed and the length of the bacteria was assessed.

We observed that the DNA replication was more inhibited in most of investigated mutant strains, in the presence of all tested metabolites; than for the control MG1655. The phenotypic effect of filamentation of bacteria was suppressed in the presence of all metabolites leading to the significantly shorter cell length. The obtained results can serve as a preliminary basis for further research on the effects of various metabolites on DNA replication and give us knowledge about the correlation between the CCM and the replication of DNA.

Key words: DNA replication, central carbon metabolism, metabolites