III.O.1

Viruses, viroids and vectors

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On San Miguel, one of the smallest of the 7000 islands of the Philippines, a few coconut palms were slowly dying in 1928. Ten years later, 50000 palms on this island were dead or dying. The disease seemed to be limited to a certain area and affected only coconut palms. No bacteria or fungi were found to be associated with the dying palms. The disease, believed to be caused by a virus, began to spread onto the nearby mainland of Luzon. It became known as cadang-cadang (dying-dying, or slowly dying, in the Bicolano language). During the following decades plant pathologists and entomologists from the USA, India, Italy and Australia were assisting their Philippine colleagues in trying to find the vector of the cadang-cadang disease agent and attempting to devise prevention and control measures.

In 1976 the disease agent was found not to be a virus, but a viroid, one of a group of plant pathogens composed entirely of a sequence of naked DNA. With the identification of the disease agent the search for vectors was abandoned. No resistant coconut palm varieties have been found. By now an estimated 50 million palms have died in and around the area of the original outbreak.

I shall describe how the cadang-cadang viroid may have been transmitted in the past and how further spread could be prevented. Until now no means for implementing control measures have been established.

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III.O.2

Initiation of *Helicobacter pylori* chromosome replication as a model to study bacterial orisome assembly on bipartite origins

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Replication of bacterial chromosome is initiated by the binding of the DnaA protein to *oriC*, which leads to DnaA oligomerisation and formation of the active orisome complex able to separate DNA helix at the DUE site and to subsequently load other replisome proteins necessary to replicate the entire chromosome. The orisome assembly is assisted by the other factors, which help to organize or regulate the initiation complex in such a way that the opening of the DUE occurs at the precisely determined moment of the cell cycle. The initiation of bacterial chromosome replication has been most deeply studied in the model organism *Escherichia coli*. However, bacteria have developed diverse modes of orisome formation and regulatory systems to adjust duplication of the genetic material to the cell cycle, which, on the other hand, depends on their life cycles and variations among the environments they inhabit. We aim at a detailed characterization of the initiation of *Helicobacter pylori* chromosome replication at the level of orisome assembly. This special emphasis on the analysis of the *H. pylori* initiation complex is justified by the bi-partite structure of its *oriC* region (*oriC1-dnaA-oriC2*), which may suggest a different mode of *H. pylori* orisome assembly than so far characterized in other bacteria such as *E. coli*. We suggest, that topology-dependent DnaA binding to *oriC2*, together with the DNA loop formed between sub-origins upon orisome assembly, may serve as a regulation strategy in *H. pylori* and complement the activity of the two so far identified proteins – HobA and HP1021 – which control orisome assembly in this pathogenic bacterium.

Key words: DnaA, *oriC*, orisome, *Helicobacter pylori*

Acknowledgements: This work was supported by a research grant from the National Science Center (SONATA BIS3 DEC-2013/10/E/NZ1/00718).

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III.O.3

Identification of a putative chromosomal replication origin from *Bdellovibrio bacteriovorus* and its interaction with the initiator protein DnaA

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Bdellovibrio bacteriovorus is a small Gram-negative, obligate predatory bacterium that attacks and invades other Gram-negative bacteria, including pathogens such as *Helicobacter pylori* or *Pseudomonas aeruginosa*. Its life cycle consists of two stages – non-replicative attack phase, wherein predator searches for prey and replicative growth phase, wherein it actively divides in host periplasm. Initiation is the first and strictly regulated step of bacterial chromosome replication, which leads to duplication of the genetic material in bacterial cells prior to their division. Our aim was to identify and characterize the key elements of initiation of chromosome replication in *B. bacteriovorus*: origin of chromosomal replication (*oriC*) and initiator protein. Using *in silico* analysis, we identified the *oriC* region, which is located downstream of the *dnaA* gene; the *B. bacteriovorus oriC* (*BdoriC*) contains eight putative DnaA boxes. Comprehensive *in vitro* studies using EMSA, DMS footprinting and SPR revealed that the DnaA protein specifically binds all eight DnaA boxes. By P1 nuclease assay we localized the DNA unwinding elements (DUE) where DNA replication starts. In addition, we compared the architecture of the DnaA–*oriC* complexes (orisomes) in homologous (*oriC* and DnaA from *B. bacteriovorus*) and heterologous (*BdoriC* and DnaA from prey, *E. coli* or *P. aeruginosa*) systems. Interestingly, we demonstrated that DnaA proteins from preys (relatively distantly related from *B. bacteriovorus*) not only specifically bind *BdoriC,* but also unwind DNA at the DUE. To conclude, we identified the *oriC* of *B. bacteriovorus* and characterize in details its interaction with the replication initiator protein.

Key words: *Bdellovibrio bacteriovirus*, DnaA, DNA replication

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III.O.4

The interplay between the actetate overflow pathway and the initiation of DNA replication in *Escherichia coli* cells

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DNA replication in *Escherichia coli* is regulated primarily at the initiation step. Timing of the initiation of DNA replication is set by the availability of DnaA protein in its ATP-bound form. It remains unclear how changes in DnaA-ATP availability are coupled to cell growth and cellular metabolism. However, previous research carried out in *Bacillus subtilis* and *Escherichia coli* revealed that mutations in certain genes of the central carbon metabolism (CCM) suppress DNA replication alterations indicating a direct correlation between DNA amplification and metabolic status of the cell. It has been demonstrated that the strongest suppression of many phenotypes of *E. coli* replication mutants is achieved by deletions of genes of acetate overflow pathway: *pta* and *ackA*. In particular, it was demonstrated that deletion of these genes suppresses temperature-sensitive growth of the *dnaA46* mutant, which produce defective initiator protein DnaA. Thus, characterization of metabolic, transcriptomic and phenotypic changes in this strains may shed light on the mechanisms coordinating the initiation of DNA replication in accordance with metabolic state of the cell.

Here we present transcriptomic and genetic data showing that in *E. coli* disruption acetate overflow pathway (encoded by *pta* and *ackA*) leads to large changes in the global transcription including significant upregulation of genes belonging to the σ^S regulon. σ^S acts as the master regulator of the general stress response in *E. coli* and in this work we show that deletion of the gene encoding σ^S (*rpoS)* abolished the suppression of *dnaA46* phenotype by mutations in CCM genes suggesting that this effect depends on the activation of σ^S regulon. Furthermore, we show that suppression of *dnaA46* thermal-sensitivity is most probably linked to accumulation of intermediate metabolites evoked by the lack of activity of acetate overflow pathway and induction of glutamate acid resistance system. Our data suggests also that disruption of *pta* and *ackA* genes leads to alterations in the control of DNA replication of strains producing the wild-type initiator protein DnaA.

Keywords: DNA replication, central metabolic pathways, σ^Sregulon, acid resistance system

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III.O.5

The lack of Hfq protein affects DNA replication process in *Escherichia coli*

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The *Escherichia coli* Hfq protein was originally described as a host factor required for bacteriophage Qβ RNA replication. Since then, variety of studies have provided us with deeper understanding of its multiple functions.
The Hfq protein act as the major global regulatory factor associated with RNA metabolism. Its riboregulatory functions have been widely described. For instance, Hfq is an RNA-chaperone that facilitates sRNAs pairing with their target mRNA and interact with proteins involved in mRNA decay. However, current studies showed that Hfq is also a DNA-binding protein associated with nucleoid.
We showed that deletion of *hfq* gene affects plasmids and chromosome DNA replication. We performed *in vivo* replication experiments by measuring the levels of radioactive thymidine incorporation during plasmid and chromosome DNA synthesis. Our results revealed differences in kinetics of replication during the bacterial growth for some replicons. Furthermore, we performed high throughput analyses. Transcriptome sequencing and phenotype microarray analysis revel shifts in genes expression as well as alterations in metabolism in *hfq* mutant.
Our findings support the previous hypothesis on the role of Hfq as the regulator of propagation of some replicons.

Key words: Hfq protein, plasmid, replication

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