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Generation of a CTO gene mutant Pseudorabies virus

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Introduction: A newly discovered nearly 300 bp long RNA coding gene called CTO-S ("close to OriL-short") was described in Pseudorabies virus (PRV) by analyzing PacBio and Illumina Hiseq full transcriptome sequencing data. CTO-S is situated between the ul22 and ul21 genes of the PRV genome and its putative promoter region is attached to the replication origin of the unique long (UL) viral genomic region. Our preliminary experiments show an abundant expression of the CTO-S transcripts which belong to the late kinetic group of PRV genes based on our Real-Time RT PCR data. To outline a concept about the function of CTO-S, we generated a mutant viral strain.

Materials & Methods: 700 bp long viral sequences around the CTO-S gene were amplified by Pfu polymerase in a GC-rich buffer system (Roche). Primer pairs with recognition sequences of restriction modification enzymes (HindIII, EcoRI) were designed to generate flanking sequences of CTO-S for the nested PCR. The 5' and 3' flanking sequences were cloned into a pBSK-lacZ gene containing plasmid. To generate an insertion/deletion mutant virus strain, a lox-CMV-GFP-lox reporter gene cassette bracketed with EcoRI recognition sites was cloned between the 5' and 3' flanking sequences of CTO-S. Mutant PRV viruses were generated by homologous recombination transfecting PK-15 cells parallel with the wild type PRV (Kaplan strain) and the reporter gene cassette.

Results: The two high GC-rich and repetitive sequence rich flanking regions of CTO-S were successfully amplified and cloned in a delivery pBSK plasmid vector. Both of them contain a HindIII linearizing restriction sites at the ends of the sequences and an insertion restriction site (EcoRI) at the middle of the construct. A CMV promoter driven GFP cassette embracing two tandem loxP sequences was inserted in two different orientations into the EcoRI site. Orientation of the reporter gene cassette was determined by traditional restriction digestion and PCR method. Activating loxP sequences by Crerecombinase produced a null mutant CTO-S PRV gene which could be further analyzed at transcriptomic level by Real Time RT PCR and sequencing.

Biography

Istvan Prazsak biologist received his diploma at the University of Szeged, Faculty of Sciences, Hungary. He worked at the Drosophila Stock Centre as student and as a PhD student at the Department of Genetics and Molecular Biology at the University of Szeged. He has worked at the Department of Medical Biology, Medical Faculty, University of Szeged as assistant research fellow as member of the Prof. Zsolt Boldogkoi's research group since 2010. His current research project is the generation of CTO mutant Pseudorabies virus with molecular cloning techniques. Beside his research Istvan is responsible for the German students at the institute.

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