Abstract of the PhD thesis of Andrey Eremeev

“Functional analysis of the parvulin protein Par14”.

In the present work the conditions for a double knockout of Pin1 and Par14 within the DT40 cell line should be established.

First the target construct for the Par14-locus was cloned in a multistep procedure. This encloses the 7 kb genomic sequence of Par14 as well as a LoxP-recognition sequence within Intron 1-2, the second LoxP place and a Neomycin-selection marker within 3' sequences. With this construct DT40-MerCreMer cells ware transfected and selected on insertion of the construct. The successful exchange of the first allele was confirmed by Southern Blot which was set up for this in the lab first. The exchange of the second allele was not successful.

The DT40 system with stable expression of the Cre-Rekombinase allows both an inducible knockout and switching off another protein (double knockout). In the present work the genomic sequence of Pin1 locus between the first and the fourth exon of *Gallus gallus* was amplified and sequenced. This was possible only after methodical improvements of the amplification and cloning of the extreme GC rich nucleotide sequences. Therefore this work gives the possibility for a later knockout of the Pin1 locus of *Gallus gallus*.

During this work for the genetic knockout of Par14 in DT40 cells a study of a working group from Japan showed, that it is possible to reduce the expression of Par14 by means of the knockdown method (Fujiyama-Nakamura et al., 2009). In the present work the expression of Par14 was clearly reduced by means of RNA interference technology with the stealth siRNA constructs. It could be shown that the knockdown of parvulin is associated with a strong change of the phenotype by the cell growth as well as the cell cycle profile. As a DNA-binding protein Par14 could also have an effect on the transkriptom. This effect was examined by microarray hybridisation. Here Par14 showed no effect which was obviously comparable with the microarrays of known transcription factors. Nevertheless, the data of the Microarray analysis deliver information for future studies to a possible compensatory function of other genes (e. g., for ribosomale biogenesis) by loss of Par14.