ABSTRACT

Cbf11 and Cbf12 proteins, the members of the CSL transcription factors family, are involved in a wide range of cellular processes in the fission yeast *Schizosaccharomyces pombe* – among other things they regulate cell adhesion and they have also been implicated in maintenance of genome integrity. At the level of the whole genome we previously identified target loci bound by CSL proteins *in vivo*. Many of them do not contain any consensus CSL-binding element. There are probably different DNA binding modes of the Cbf11/12 proteins and it has not been known what specific biological function is associated with the particular way of DNA binding.

For the purpose of studying CSL DNA binding modes we have worked in this project on the implementation of the DNA binding mutation (DBM), which prevents direct DNA binding of CSL proteins to canonical motif *in vitro*, into the chromosomal locus of the *cbf11* and *cbf12* genes. Using the "*ura4* selection system" we have successfully constructed the scar-less Cbf12-TAP and Cbf12DBM-TAP knock-ins, i.e. the strains without/with DBM in the open reading frame of Cbf12 where Cbf12 is C-terminally TAP-tagged and contains the intact 3'UTR. In our laboratory we have established the CRISPR/Cas9 system by which we have been able to prepare the Cbf11-TAP strain. We have failed to construct the Cbf11DBM-TAP strain, but we discuss some possible optimizations of the construction process that could lead to a successful result. Based on the results obtained by the washing assay we have found out that the functional DNA binding domain of the Cbf12 is necessary for the regulation of cell adhesion. These results correlate with the data from binding study where we observed the binding of Cbf12 vs. Cbf12DBM *in vivo* to the promoter regions of the *cbf12*, *gsf2* and *pf17* genes involved in the regulation of cell adhesion. Our results further show that the ability of canonical direct DNA binding of Cbf12 is not required for the role of Cbf12 protein in the process of maintaining genome integrity. DBM mutation is a useful tool to elucidate DNA binding modes of CSL proteins in *S. pombe*.

Keywords:

Schizosaccharomyces pombe, transcription factor, CSL proteins, Cbf12, DNA binding, DNA binding mutation (DBM), adhesion, genotoxic stress