Abstract

CSL (CBF1/RBP-Jκ, Suppressor of Hairless, Lag-1) protein family members are transcription factors critical for metazoan development as the effectors of Notch signaling pathway as well as in a Notch-independent manner. CSL homologues have been identified in fungal organisms lacking the Notch signaling pathway. Cbf11 and Cbf12 are antagonistic paralogous proteins that are important for proper coordination of cell and nuclear division, regulation of cell adhesion and chromosome integrity in the fission yeast *Schizosaccharomyces pombe*. The activities of Cbf11 and Cbf12 proteins need to be finely balanced for their proper biological function, however, chromosomally tagged alleles of these proteins exhibit properties different from wild type. Therefore, the availability of specific antibodies would greatly enhance the study of CSL proteins in the fission yeast. In this bachelor's thesis, design and preparation of imunogen for commercial antibody production followed by antibody testing is presented. Using bioinformatics, suitable immunogenic Cbf11 and Cbf12 protein fragments were selected and the corresponding DNA sequences were cloned into an expression vector. His-tagged expression was optimized in a bacterial expression system and the native protein was purified using immobilized metal affinity chromatography. Rabbit polyclonal antibody against the prepared protein was commercially acquired. The sensitivity and specificity of the polyclonal antibodies raised were validated using Western blot. Both fission yeast CSL paralogues were recognized by the prepared polyclonal antibody, however, only the TAP-tagged proteins were detected. Interaction of the antibody with the endogenous untagged CSL proteins was not found, likely due to low copy number of these proteins.