# CSL transcription factors: indirect binding to DNA in fission yeast?

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## Introduction

The members of the CSL transcription factor family are part of the Notch signalling pathway in metazoans. While the Notch pathway appeared during the evolution of animals, CSL proteins are evolutionarily older and have been identified also in fungi (Převorovský *et al*., 2007). The fission yeast CSL proteins are important for the proper coordination of cell cycle events and regulate cell adhesion (Převorovský *et al*., 2009). Using the genome-wide ChIP-seq technique, we have identified loci bound *in vivo* by Cbf11 and/or Cbf12, the fission yeast CSL proteins. We noticed different classes of binding events. As expected, target loci were found in the regulatory regions of genes, but they also occurred in the intergenic regions and ORFs. Moreover, many of them did not contain CSL-binding element. It suggests that CSL proteins bind to target loci directly and possibly also indirectly, i.e. via other DNA-binding interaction partners.

## Aims of the project

1) to test whether CSL proteins bind to particular target loci directly or indirectly
2) to find out what CSL roles are associated with direct and indirect binding to DNA, respectively

## The approach we took

We will determine the way of binding by using a point mutation introduced into the DNA binding domain of CSL proteins (DBM = DNA binding mutant). The amino acid substitution does not affect stability of the protein, but the protein loses its ability to bind DNA directly (Oravcová *et al*., 2013).

For monitoring of DNA binding of Cbf11/12DBM proteins under physiological conditions we need to prepare the strains that would include (Fig. 1):

- DBM in the CSL chromosomal locus (Cbf11DBM - R318H; Cbf12DBM - R644H)
- TAP/HA tag behind the 3' end of the ORFs
- intact 3'UTR

## How we did it:

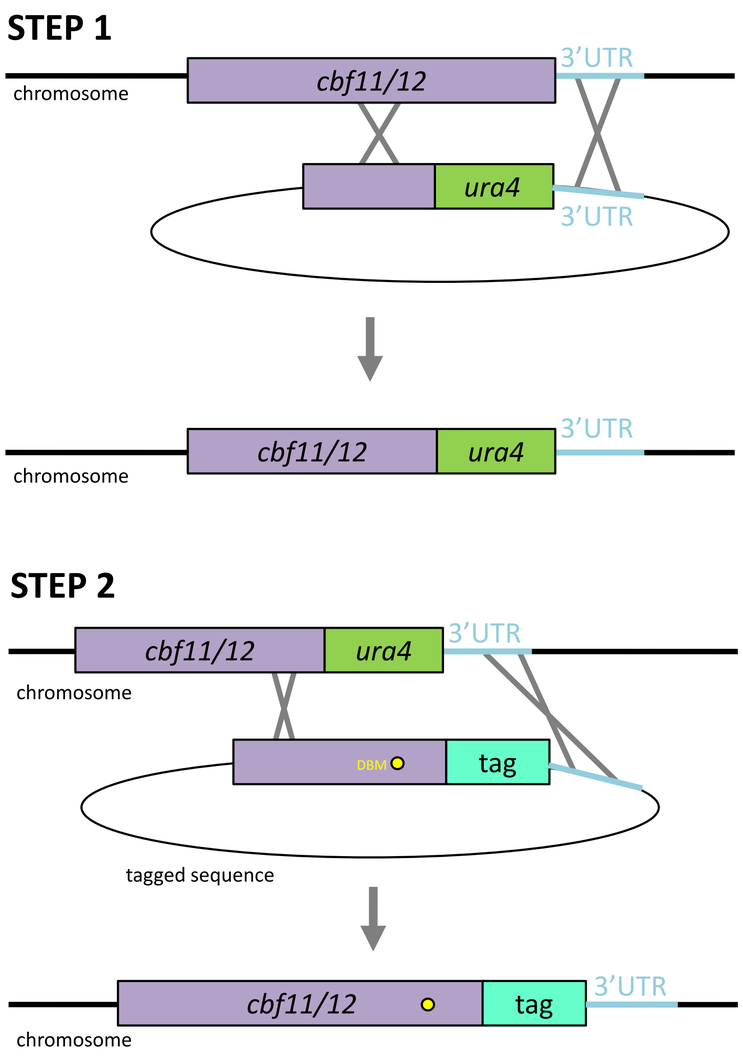


**Figure 1.** Marker replacement method (*ura4* selection system) - schematic model of the approach we took.

## Acknowledgements

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## Results so far

We constructed C-terminally TAP-tagged Cbf12 and Cbf12DBM knock-ins, both with an intact endogenous 3'UTR. The constructs were verified by PCR, sequencing and Western blot (Fig. 2).

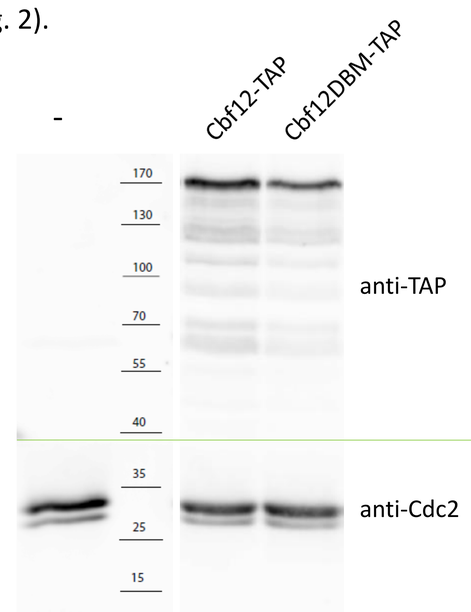


**Figure 2**. New strains Cbf12-TAP and Cbf12DBM-TAP validated by Western blot.

We measured the growth rate and calculated doubling time ($T_d$) of created strains Cbf12-TAP and Cbf12DBM-TAP. The preliminary data indicate that DBM mutation slows growth rate (Table 1).

| | Wild-type | Cbf12-TAP | Cbf12DBM-TAP |
|---|---|---|---|
| $T_d$ (min) | 128 | 134 | 143 |

**Table 1**. Doubling time ($T_d$) of Cbf12-TAP and Cbf12DBM-TAP.

We did a pilot ChIP-qPCR study of binding of wild-type Cbf12 and its DBM variant to a panel of previously characterized target loci (Fig. 3).

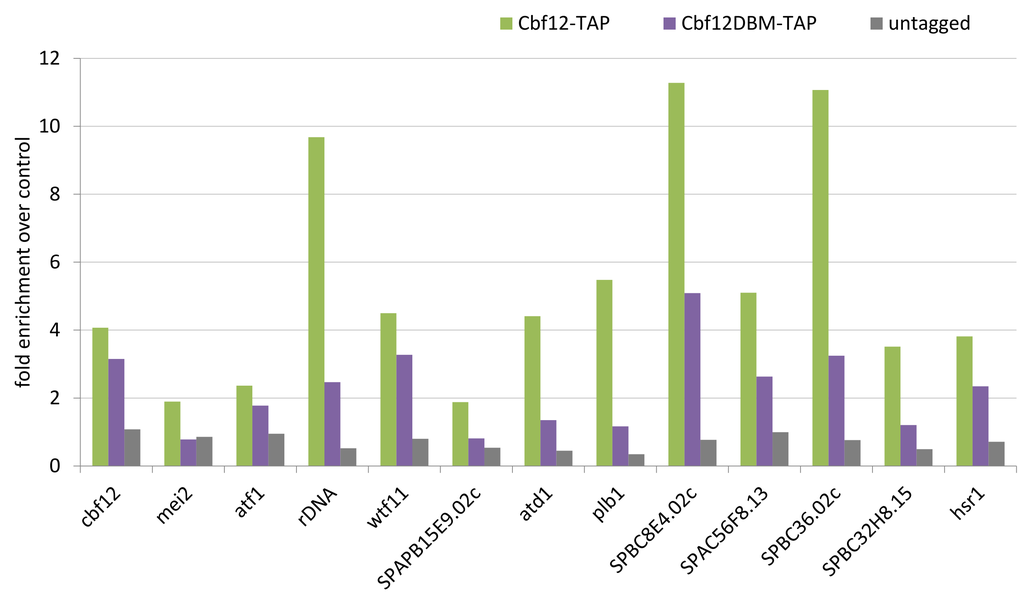


**Figure 3**. A pilot ChIP-qPCR study of binding of Cbf12-TAP and Cbf12DBM-TAP to target loci. Binding to DNA was affected by the DBM mutation at different sites to varying degree.

## Future steps

- to construct Cbf11 or Cbf11DBM knock-ins with C-terminal TAP tag and intact 3'UTR using CRISPR-Cas9 system (Jacobs *et al.*, 2014), because we failed to prepare these strains using the *ura4* selection system
- to study the binding of Cbf11 and its DBM variant to target loci using ChIP-qPCR
- to determine which CSL regulated processes are associated with direct binding of CSL proteins to DNA and in which processes the direct binding is not required

## References

Jacobs, J. Z., Ciccaglione, K. M., Tournier, V., & Zaratiegui, M. (2014). Implementation of the CRISPR-Cas9 system in fission yeast. *Nature Communications*, *5*, 5344.

Oravcová, M., Teska, M., Půta, F., Folk, P., & Převorovský, M. (2013). Fission yeast CSL proteins function as transcription factors. *PloS One*, *8*(3), e59435.

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