# Experimental tools to determine DNA binding sites of KRAB zinc finger proteins in their candidate target genes – a challenge in computational biology of transcriptional regulatory networks

**Peter Lorenz[1], Sabine Dietmann[1], Christian Sina[1], Dirk Koczan[1], Steffen Möller[1] and Hans-Juergen Thiesen[1, 2]**

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## 1 Introduction.

The ordered expression of genes in transcriptional networks is an important means of living organisms to control biological processes. Transcription factors play pivotal roles in such networks. C2H2 zinc finger proteins form one of the largest protein superfamilies in the human genome with more than 1300 members according to INTERPRO (domain IPR007087). Typically, these proteins contain an array of multiple C2H2 domains that is thought to specify nucleic acid binding and also contribute to protein-protein interactions. One particularly interesting subclass of the C2H2 zinc finger proteins contains the Krueppel-associated box (KRAB; INTERPRO domain IPR001909; more than 400 human members listed) domain that confers potent transcriptional repression activity upon targetted promoters (Thiesen, 1990; Margolin et al., 1995). We have started to combine experimental strategies for the search of target genes with bioinformatic approaches to define target gene signatures and DNA binding motifs of KRAB zinc finger proteins. The challenges to define such signatures and motifs reside in the degenerate binding code of individual zinc fingers, the potential different binding site specificities in a multi-zinc finger array in different parts of the protein and the fact that KRAB-mediated repression can also be elicited from remote positions with respect to the regulated gene.

## 2 Materials and Methods.

The target detection assay. (TDA; Thiesen and Bach, 1990) employed random 15mer double-stranded oligodeoxynucleotides and recombinant KRAB zinc finger proteins Kox1/ZNF10 (X52332) to enrich for DNA sequences with high binding affinity and to define respective binding matrices. A variation of this approach employed genomic fragments from a PAC clone containing C2H2 zinc finger genes instead of oligonucleotides. The impact of ectopic overexpression of KRAB zinc finger proteins on the global RNA expression profile of cultured human HeLa cells was recorded. Genes influenced in their transcriptional activity by this overexpression should contain direct targets of the KRAB zinc finger proteins as well as genes that are affected as secondary reactions, e.g. in gene networks. Antisense oligonucleotides were employed to downregulate intracellular expression of Kox1 in HeLa cells. Then concomitant changes in RNA expression profiles were monitored. Potential target genes should be to a certain extent relieved from Kox1-mediated repression and thus increase in their expression. Bioinformatics tools have been developed to evaluate and to combine the results of the experimental procedures.

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[1] Institute of Immunology, Proteome Center Rostock; www.pzr.uni-rostock.de;
University of Rostock, Schillingallee 70, D-18055 Rostock, Germany
[2] E-mail: hans-juergen.thiesen@med.uni-rostock.de

# 3 Results.

Oligonucleotide sequences derived from the TDA selection were initially compared with each other to define DNA binding preferences of KRAB zinc finger proteins. In a second TDA approach, randomised oligonucleotides were replaced by a PAC clone encoding zinc finger gene sequences on human chromosome 10. Furthermore, zinc finger specific target genes were identified to be induced or repressed in HeLa cells with ectopically expressed zinc finger proteins. Finally, target genes were determined by KRAB zinc finger genes that had been inactivated by antisense-oligonucleotides. Sequence information of all four distinct approaches were taken to determine putative binding sites by making use of novel software tools. Affinity selection of recombinant Kox1 protein by the TDA resulted in 31 15mer oligonucleotide sequences that did not lead to a homogeneous consensus binding matrix. Since Kox1 contains nine functional C2H2 zinc fingers and each finger potentially contacts 3 nucleotide residues, a panel of 15mers is not sufficient in length to cover all putative binding sites offered by Kox1. Thus, binding activities within the same protein are most likely competing for the oligonucleotides being selected leading to the inhomogeneity observed. Indeed, taking into account the binding frequencies to 3-6mer sequence patterns argued for specific sequence preferences that could be found as well in the genomic DNA sequences selected by the TDA. Following the downregulation of Kox1 gene expression by specific antisense oligonucleotides 81 out of 44928 probed gene sets on Affymetrix microarrays were increased in their RNA levels. These genes constitute a Kox1 candidate target gene list in HeLa cells. Overexpression of Kox1 in HeLa cells did result in the increase of 32 and the decrease of 30 gene transcripts in their abundance. However, none of the genes were also found on the candidate target gene list after downregulation of Kox1 expression by the antisense approach. By applying sophisticated software tools the distribution of putative Kox1 DNA binding sequences were evaluated within the genomic sequences of Kox1 candidate target genes. Seed combinations of the double-stranded oligonucleotides selected by KOX1 proteins led to the identification of sequence motives within the original PAC DNA. Finally, these motives could be determined in target genes detected by Affymetrix microarray analysis as well.

# 4 Discussion.

The usefulness of our strategy is based on the combination of in-vitro selected DNA binding sequences with target gene signatures in vivo that were the result of perturbed KRAB zinc finger gene expression employing bioinformatic tools. However, the quality and robustness of bioinformatic information has still to be validated and confirmed in experimental settings – a challenge for developing more sophisticated algorithms mimicking DNA-protein interactions of KRAB zinc finger genes.

# 6 References and bibliography.

# References

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