COMPUTATIONAL MODELLING IN MEDICINE

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Cooperativity between Aryl Hydrocarbon Receptor and β-catenin binding sites in CYP1A1 Induction

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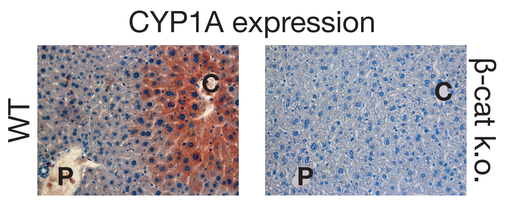
ABSTRACT

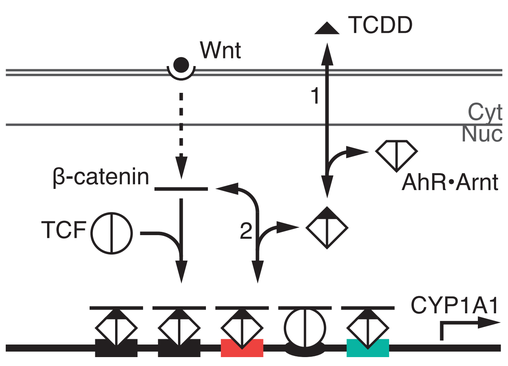
Cellular responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or similarly acting compounds are mediated by the aryl hydrocarbon receptor (AhR). Upon activation by appropriate ligands a heterodimer of AhR and its nuclear translocator Arnt act as a transcription factor (TF) at dioxin response elements (DREs) in the promoter region of target genes such as Cytochrome P450 1A1 (CYP1A1). In addition, signaling through the Wnt/β-catenin pathway has been identified as a modulator of AhR-induced hepatic CYP1A1 expression. The mechanisms underlying the interplay between these different TFs converging on the CYP1A1 promoter are still poorly understood. With the help of in vitro mutation studies of the human CYP1A1 promoter we developed a mathematical model that combines a simplified view of the Wnt/β-catenin signaling pathway with a statistical mechanical description of transcription factor binding. This two-tier model is able to reproduce the cooperative effects between the promoter binding sites and seen in the data and qualitatively predict the portocentral gradient of CYP1A1 expression in the liver. Statistical mechanical modeling has the capacity not only to unravel the interaction of transcription factors on a promoter level but also to predict protein expression on the scale of a liver lobule.

BACKGROUND

With the help of the xenobiotic metabolism drugs and poissons are removed from the body. As one of the main proteins involved, Cytochrome P450 1A1 (CYP1A1) is expressed around the central hepatic vein upon exposure to TCDD. The comparison of WT hepatocytes with ones in which β-catenin is knocked out gave us a first hint that there exists a link between the Wnt signaling and the AhR mediated xenobiotic metabolism.

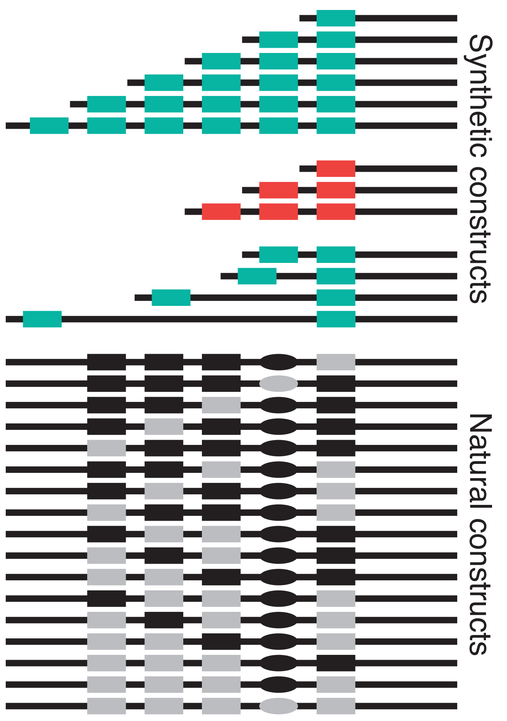


Both, the Wnt and the AhR signaling pathway had been observed to converge on the CYP1A1 promoter on which there exist 5 functional transcription factor binding sites (TFBS) - 4 are targeted by AhR-Arnt heterodimer, 1 is bound by TCF/Lef.



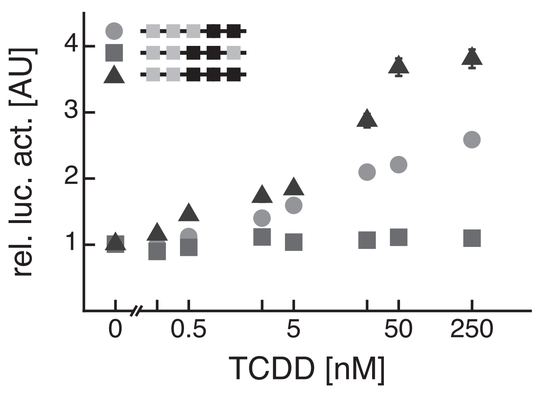
MATERIALS & METHODS

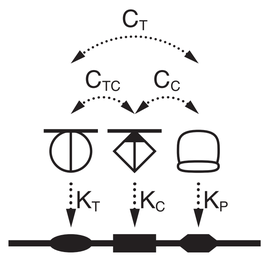
To analyze cooperative interaction between the TFs we constructed a library of mutant promoter constructs.



RESULTS

1. Cooperativity can be analysed with a statistical mechanics model



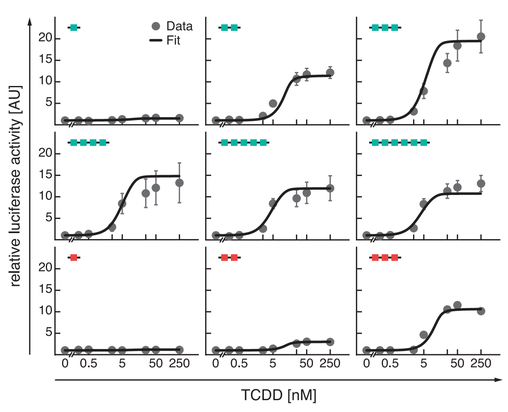


$$p^{FC}_{bound} = \frac{\frac{[P]}{K_P} + 1}{\frac{[P]}{K_P} + \frac{1}{F_{reg}}}$$

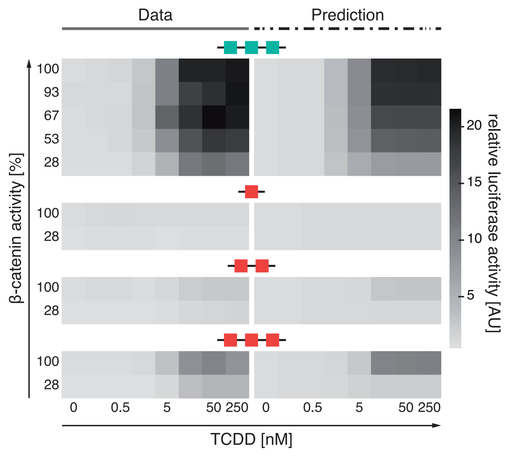
$$F_{reg} = \frac{1 + \frac{[C]}{K_C} C_C + \frac{[T]}{K_T} C_T + \frac{[C]}{K_C}\frac{[T]}{K_T} C_C C_T C_{CT}}{1 + \frac{[C]}{K_C} + \frac{[T]}{K_T} + \frac{[C]}{K_C}\frac{[T]}{K_T} C_{CT}}$$

We found more-than-additive effects between TFs that bind to the dioxin responsive elements (DREs). It has been shown previously than such effects can be described with the help of a statistical mechanical fold-change model as depicted above. Taken together with a signaling model that describes TF formation (see Background), we set off to unravel this behavior in more detail.

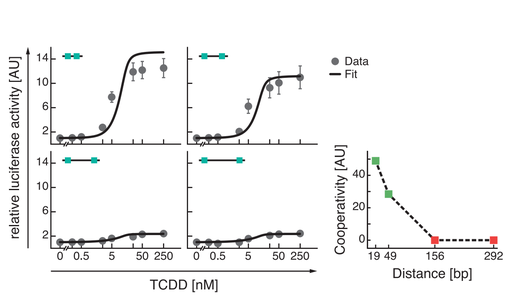
2. Synthetic promoter constructs



The model is able to describe to behavior very well and even has predictive powers when the constructs are additionally treated with an β-catenin inhibitor.

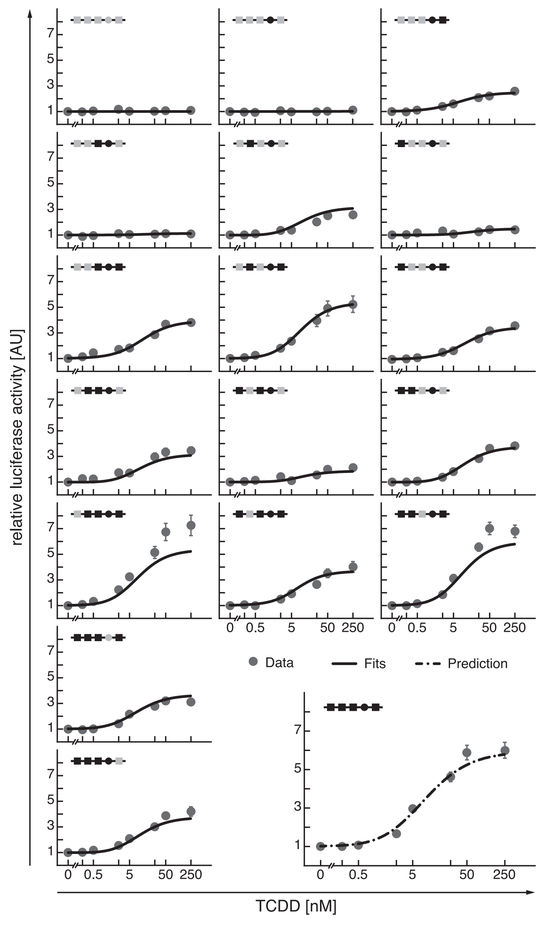


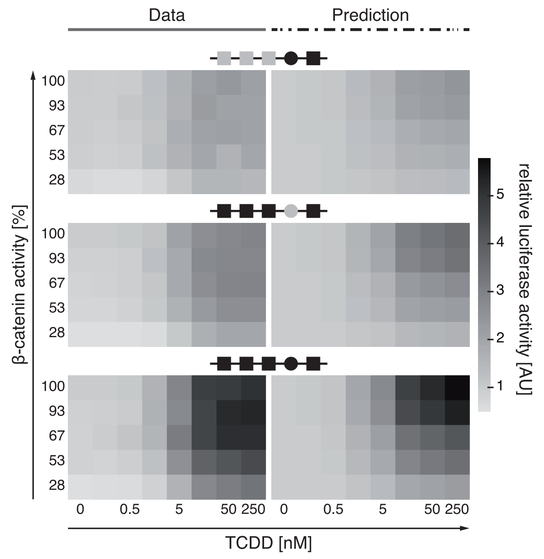
We also observe that the distance between the TFs has a strong effect on the strength and type of cooperativity.



3. Natural promoter constructs

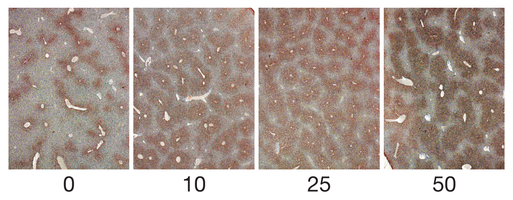
The model of the natural promoter inherited some parameters from the synthetic promoter model and is again able to describe the TCDD concentration series of the mutant promoter constructs very well. Furthermore, we were able to predict the wild-type induction and the two-dimensional data. It can also be observed that the AND gate like properties of the data were also replicated quite well.





OUTLOOK

In a next step we will be able to predict the CYP1A1 expression gradient in liver lobules.



CONCLUSION

The effects of transcription factor cooperativity on expression patterns on a physiological scale was previously hard to determine. With the help of a two-tier model consisting of a signaling part and a statistical mechanics part, we were able to not onyl describe the induction of CYP1A1 in hepatocytes but also predict the expression pattern when the activity of β-catenin was inhibited additionally.

AFFILIATIONS // FUNDING

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