COMPUTATIONAL MODELLING IN MEDICINE

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Signal Integration by the *CYP1A1* Promoter — A Quantitative Study

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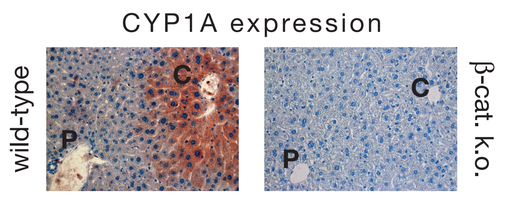
ABSTRACT

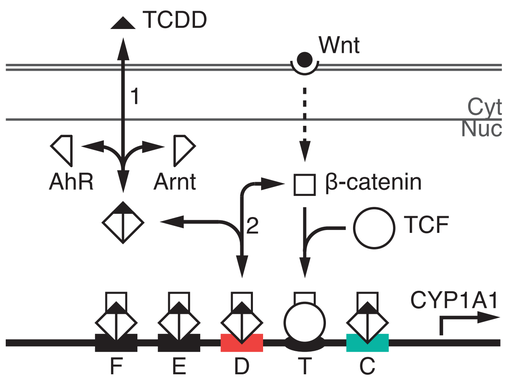
Genes involved in detoxification of foreign compounds exhibit complex spatiotemporal expression patterns in liver. Cytochrome P450 1A1 (*CYP1A1*), for example, is restricted to the pericentral region of liver lobules in response to the interplay between aryl hydrocarbon receptor (AhR) and Wnt/β-catenin signaling pathways. However, the mechanisms by which the two pathways orchestrate gene expression are still poorly understood. With the help of 29 mutant constructs of the human *CYP1A1* promoter and a mathematical model that combines Wnt/β-catenin and AhR signaling with the statistical mechanics of the promoter, we systematically quantified the regulatory influence of different transcription factor binding sites on gene induction within the promoter. The model unveils how different binding sites cooperate and how they establish the promoter logic; it quantitatively predicts two-dimensional stimulus-response curves. Furthermore, it shows that crosstalk between Wnt/β-catenin and AhR signaling is crucial to understand the complex zonated expression patterns found in liver lobules. This study exemplifies how statistical mechanical modeling together with combinatorial reporter assays has the capacity to disentangle the promoter logic that establishes physiological gene expression patterns.

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/β-catenin signaling and the AhR mediated xenobiotic metabolism.

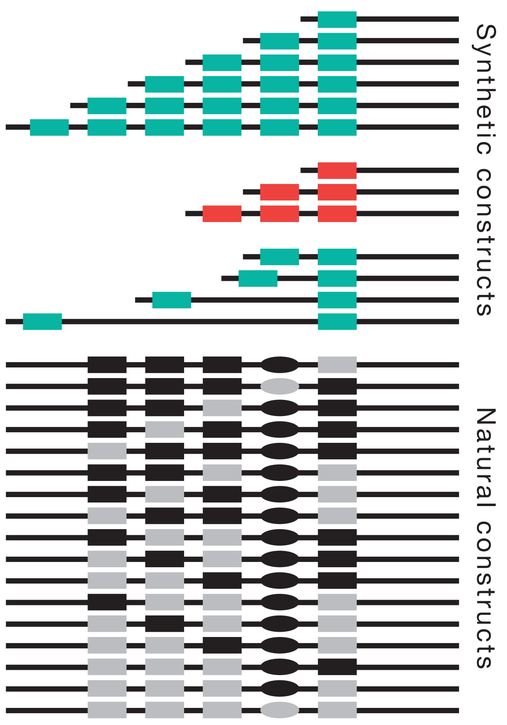


Both, the Wnt/β-catenin and the AhR signaling pathway had been observed to converge on the *CYP1A1* promoter on which there exist five functional transcription factor binding sites (TFBS) - four are targeted by AhR-Arnt heterodimer (DREs) and one is bound by β-catenin/TCF.



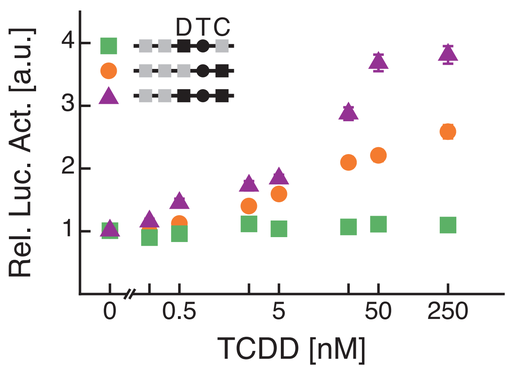
MATERIALS

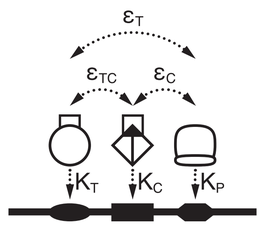
To analyze cooperative interaction between the TFs we constructed a library of mutant promoter constructs.



RESULTS

1. Cooperativity can be analysed with a statistical mechanics model



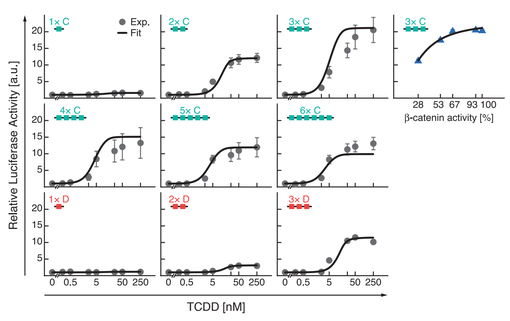


$$\phi_M = \frac{\frac{[P]}{K_P} + 1}{\frac{[P]}{K_P} + F_{\mathrm{reg}}}$$

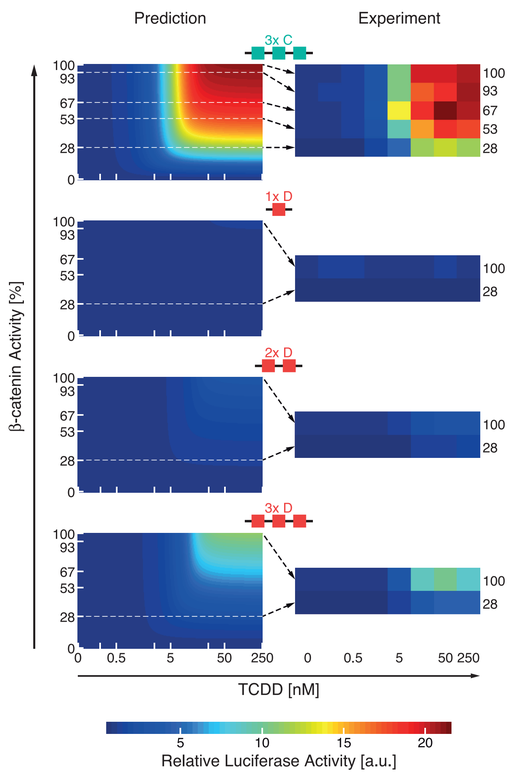
$$F_{\mathrm{reg}} = \frac{1 + \frac{[C]}{K_C} + \frac{[T]}{K_T} + \frac{[C]}{K_C}\frac{[T]}{K_T}\epsilon_{CT}}{1 + \frac{[C]}{K_C}\epsilon_C + \frac{[T]}{K_T}\epsilon_T + \frac{[C]}{K_C}\frac{[T]}{K_T}\epsilon_C\epsilon_T\epsilon_{CT}}$$

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 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.

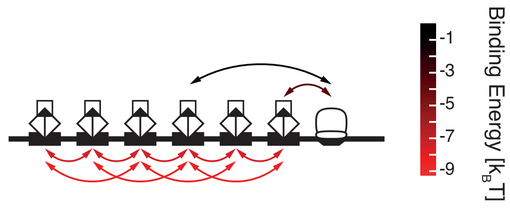
2. Synthetic promoter constructs



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

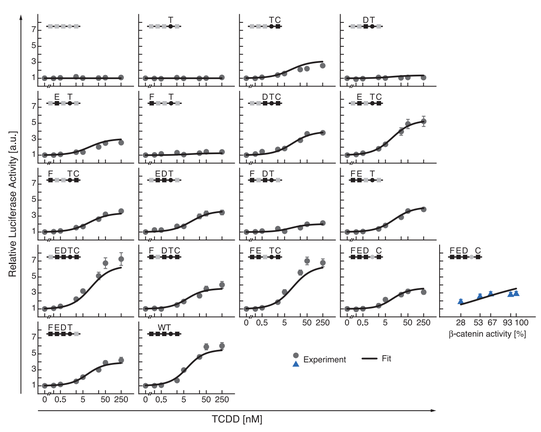


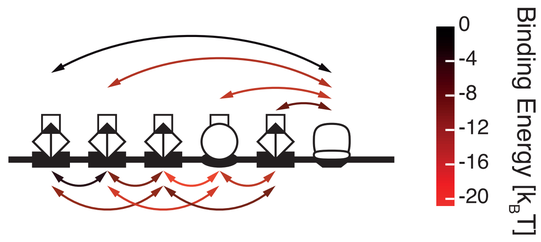
The parameters exhibit a strong cooperativity between DREs while only the first DRE interacts strongly with the RNAP.



3. Natural promoter constructs

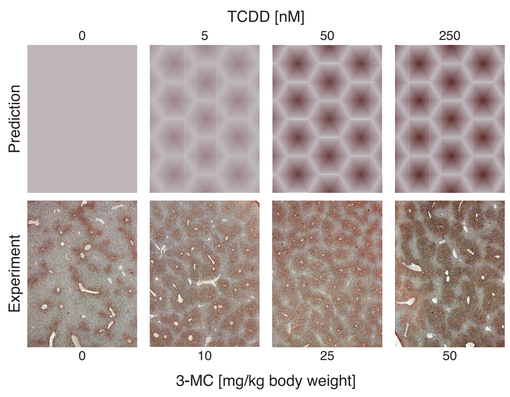
The model of the natural promoter inherited some parameters from the synthetic promoter model and is again able to describe the TCDD concentration series of the mutant promoter constructs very well. Furthermore, we were able to predict the double-stimulated data. It can also be observed that the AND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

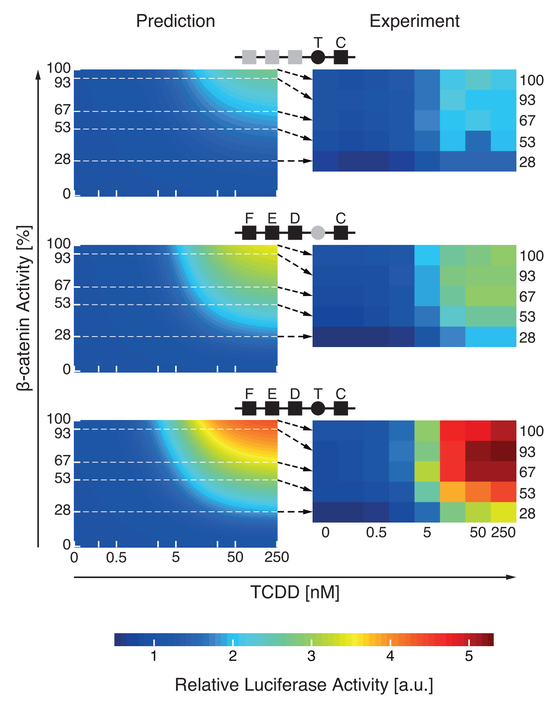




4. Prediction of the Physiological Hepatic Zonation Patterns

The model is able to predict the spatial expression of *CYP1A1* as a result of its promoter logic and the interaction of the upstream signaling pathways.





CONCLUSION

- Wnt/β-catenin and AhR signaling interact via binding and convergance on the *CYP1A1* promoter
- More-than-additive cooperativity between C- and D-DRE
- DREs in synthetic constructs interact almost exclusively via cooperation
- Sequestration responsible for reduced transcriptional induction.
- Logic AND gate connects the Wnt/β-catenin and AhR signaling pathways
- Model is able to predict double-stimulated data of synthetic and natural promoter mutants
- Model is able to predict the physiological zonation pattern in liver lobules

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