Probing different thermodynamic models of transcriptional gene regulation in case study of even-skipped gene regulatory region in *Drosophila*

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Mathematical models based on the statistical thermodynamics provide a promising approach for modeling gene regulatory networks at the sequence level. This approach was successfully tested on many biological systems. An example is a regulatory region of the *Drosophila* even-skipped gene expressing during the early embryonic development [1]. A sequence-based model was applied in [1] to simulate the reporter gene expression under control of 1.7kb-length even-skipped upstream regulatory sequences. Results of the modeling include specific predictions about binding sites distribution in the control region and their relative strengths in terms of transcription factor (TF) binding and in terms of the influence on the target gene expression. Our aim was to verify these predictions by applying an alternative modeling approach to the same biological system. This study has a general purpose to investigate robustness (model dependency) of model predictions about the role of regulatory sequences in gene expression control. We used a thermodynamic model proposed in [2] as the alternative modeling approach and fitted this model to the same expression data as in [1]. This model is more flexible in varying mechanisms of transcriptional regulation and, thus, allows to test the biological system in a range of different model assumptions. We found that the alternative model was able to produce solution for the target gene expression of the same quality as the original model. However, new values for the model parameters (binding affinities for the binding sites and the regulatory action strengths for the TFs) exhibited high variability in the set of the fitting experiments, indicating that these values have rather low confidence. We quantified the significance (or strength) of each of the 34

binding sites in the regulatory region in two ways. The strength of a site to bind a TF is naturally described by its affinity constant. However, this strength is not always correlated with the strength of the influence that the site exerts on the transcription rate of the target gene. We estimated the latter in both models by calculating the magnitude of the expression pattern change after excluding the site from the model. We showed that the site strength estimates of both types do not correlate in the two models. This result indicates that the models give different predictions about the role of the binding sites in the transcriptional regulation. Finally, we tested two possible mechanisms for transcriptional repression (by short-range quenching of activator molecules and by direct inhibition of the basal transcription machinery). The computations revealed that the direct inhibition model had the same solution quality as the model with the short-range repression, confirming a similar conclusion obtained in [2] for a different biological system. The results obtained in our study raise questions about general applicability of the thermodynamic models for dissecting the 'regulatory architecture' of the transcriptional enhancers. Conclusions about the importance of various binding sites in a regulatory sequence in the context of both their binding ability and their influence on the expression can be model dependent, as it happens in the case of the even-skipped regulatory region considered in the study.

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