Transcription regulation in time and space: Engineered cell systems to modulate the epigenetic chromatin structure: The role of Methyl-CpG-binding protein 2

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Publication date
2016

Document Version
Final published version

Summary

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Transcription processing and chromatin structural changes are intertwined processes in the nucleus. In this thesis we focused on the dynamic interplay of chromatin and its widespread functions such as genome stability and transcription regulation. We studied the effect of targeting the epigenetic regulatory protein Methyl CpG binding protein2 (MeCP2) and measure alterations in chromatin structure and and transcription regulation in real-time, in order to unravel the causal relationship between the epigenetic chromatin state and transcriptional repression in time and space.

In **the introduction** we introduce transcription regulation, chromatin and epigenetics and describe the molecular interactions of transcription. We introduce MeCP2 and neurodegenerative diseases related to this protein. We conclude with an explanation of using engineered reporter gene arrays in cultured cells, a method used throughout this thesis in order to modify chromatin and subsequently determine cause and consequence of alterations in chromatin structure and/or transcriptional activity.

In **Chapter 1** we use an experimental set-up that allows us to modulate the chromatin context (lacO and tetO targeting) of a reporter gene array and to measure the decrease in MS2 tagged transcripts in real-time in single living cells. We modulate the chromatin context by targeting methyl-CpG binding protein 2 (MeCP2). Our data show that the measured transcription repression follows a biphasic behavior consisting of a time-delayed response and a subsequent exponential decrease in the transcripts at the reporter gene array that is rather time-consuming compared to the rapid decrease in transcriptional activator at the array. The delay time preceding the decrease in transcript levels is significantly shorter when MeCP2 is targeted to the reporter gene array. We simulated the diffusion rate of MS2 tagged transcripts with a 2 dimensional finite difference diffusion model. These data estimate that mRNA diffusion from the reporter gene is much faster than the measured decrease in transcripts at the array. Our data indicate that transcription initiation is still ongoing beyond the point that the transcriptional activator is removed from the reporter gene array with a delayed response. The MeCP2-targeted chromatin context only affects the response time and not the actual decrease in transcripts, suggesting that the local chromatin context determines

whether new transcription initiation events are allowed thereby regulating the delay time of transcription repression.

In **Chapter 2** we describe the design and construction of a cell line harboring a novel reporter gene cassette. We designed this reporter gene cassette such that it can be integrated via homologous integration as a single known genomic integration site. The cassette is equipped with a readout to measure mRNA and protein kinetics of a reporter gene in real-time upon modulating the epigenetic chromatin state. The bacteriophage MS2 hairpin repeats are used to detect RNA transcripts via fluorescently tagged MS2 protein binding. We used a fluorescent protein consisting of a degradation signal coding for a short-lived fluorescent protein allowing to measure changes in protein level of the reporter gene upon inducing transcriptional repression in time. The integration of a 84x tetO tandem repeat upstream of the reporter gene enables modulation of the epigenetic chromatin state via tetR targeting. We created tetR-tagged epigenetic regulatory proteins MeCP2 and HP1 to study reporter gene transcript and protein levels at single gene level in real-time in living cells upon inducing a repressive chromatin context. We show that the reporter gene cassette is being expressed when transfected in human cells and that the target constructs bind to their tet operator repeat binding sequences. In future studies will integrate the cassette via homologous integration in the human genome and measure single gene, single cell real-time transcription upon modulating a defined chromatin structure.

In **Chapter 3** we provide an overview of the numerous functions of MeCP2 contributing to transcriptional regulation and chromatin organization. The disordered protein structure of MeCP2 allows MeCP2 to bind with its MBD, TRD or C-terminal domain to various DNA sites and regulatory proteins. Dependent on the posttranslational modification state of MeCP2 and on its binding partners, MeCP2 is able to perform opposing tasks such as transcriptional repression or activation. We highlight the molecular effects of MeCP2 (dys)regulation at the cellular level; Loss and gain of function of MeCP2 is catastrophic for the identity of brain cells and gives rise to neurodevelopmental diseases such as Rett and Xq28 duplication syndrome. We provide a model-representation showing how the

delicate balance of the affinity of MeCP2 binding and the concentration of MeCP2 afffects the MeCP2 functional fraction. Our model-representation indicates that the molecular behavior of Rett syndrome and Xq28 duplication syndrome are strikingly different although their disease symptoms show quite some overlap.

In **Chapter 4** we provide evidence that targeting MeCP2 to a large amplified chromosomal lacO array elicits an as yet undiscovered extensive chromatin unfolding. This chromatin unfolding is also observed, but to a lesser extent, when untargeted MeCP2 is overexpressed. We quantified the MeCP2-induced chromatin unfolding of full length MeCP2 versus its partial domains, i.e. MBD, TRD, C-terminus, MeCP2 lacking its C-terminus, or the Rett mutant MeCP2. For chromatin structure measurements, 3D confocal microscopy images of the large amplified chromosomal lacO array were used to determine changes in 3D image analysis parameters of the chromosomal array, i.e. the surface factor which represents the surface of a given chromosomal domain or object normalized to the surface of a sphere with an equal volume. We noted that none of the partial MeCP2 domains alone were capable of inducing the extensive chromatin unfolding produced by full-length MeCP2. FLIP analysis showed that MeCP2 binding triggers the loss of HP1γ from the chromosomal domain and an increased HP1γ mobility in the cell nucleus, which is not observed for other HP1 isoforms HP1α and HP1β. MeCP2-induced chromatin unfolding is not associated with transcriptional activation. In relation with these observations, we describe in chapter 2 that MeCP2 targeting to a reporter gene array diminished the delay in transcription repression response while the subsequent decrease in transcripts at the reporter gene array appeared unaffected. Based on these findings we proposed that the local chromatin context determines whether new transcription initiation events are allowed thereby regulating the delay time of transcription repression. Our findings in chapter 5 might indicate that MeCP2-induced chromatin unfolding prepares chromatin for subsequent transcriptional regulation, facilitating a switch in gene activity.

The causal relationship between the molecular interactions underlying protein kinetics, chromatin structural alterations and transcriptional activity is a central challenge in the field and biochemically difficult to determine. The use of engineered cell systems allows us to focus on a defined set of involved molecular interactions within the context of a living cell and modulate their actions in time and space. Such an engineered approach enables to test and demonstrate complex temporal regulation at single molecule, single cell and genome-wide levels enhancing our understanding of the concerted actions of transcription dynamics in vivo. In this thesis we designed a single cell, single gene set-up integrating the reporter gene cassette via homologous recombination.

Nowadays CRISPR/Cas9 molecular engineering provides many opportunities to create genomic changes and follow molecular interations of transcription regulation at endogenous genes and in a modified epigenetic context. CRISPR/Cas9 engieering opens-up a novel field with infinite potential for cell engineering. Another recent advance in the field is determining gene activity regulation biochemically in single cells at DNAsequence resolution level. Such single cell genome-wide analysis enables to robustly profile RNA, DNA methylation and chromatin folding but it does not give insight in temporal and spatial aspects of transcription regulation and the epigenetic chromatin state.

These biochemical population apporaches have been complemented with single cell imaging techniques. Despite tremendous advances in our understanding based on these biochemical and single cell microscopical studies, we still lack mechanistic insight of molecular concerted actions of transcription regulation in time and space.

In this thesis we focus on MeCP2, an epigenetic regulatory protein well known from MeCP2 related diseases caused by mutations in the MeCP2 coding region. We discuss the various different functionalities of MeCP2, i.e. to act as an activator as well as a repressor. Moreover, we conclude that determining molecular functioning of MeCP2 is essential to find ways to monitor or treat MeCP2 related disease development. In our reporter gene array studies we show that MeCP2 is able to alter chromatin structure and timing of transcription repression. These data suggest that MeCP2 acts as a 'facilitator' protein being able to either induce gene activity or silencing based on the facilitated sequence of

events. This facilitator functioning might be a general concept of the concerted action of epigenetic regulatory proteins to establish and maintain transcription patterns.