Understanding gene expression variability in its biological context using theoretical and experimental analyses of single cells

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Chapter 7

General Discussion

Understanding the molecular principles of gene expression regulation and variability is a major focus of fundamental life sciences. Many questions are still unresolved. For example, what determines gene expression states of (isogenic) cells and to what extent is this affected by expression levels of single cells. Answers to these fundamental questions is essential to understand how cells function and how their behavior changes in response to alterations in their environment. In this thesis we first analyzed how cellular volume (a function cell growth) impacts gene expression variability. Next, we studied gene expression changes after inducing altered cell states by (i) chromatin remodeling, (ii) exposure to UV and (iii) by longterm exposure to drug treatment (hormonal treatment in breast cancer cells).

7.1 Biologically relevant variation in gene expression is best captured by concentration variability

Traditionally, variability in gene expression is quantified by measuring absolute mRNA copy numbers [29, 50, 63]. However, since reaction rates inside cells are determined by concentrations of molecules, concentration variability is an improved measure of biologically relevant variation. By combining single cell mRNA and volume measurements, we quantified single-cell mRNA concentration variability. We demonstrate that the measured mRNA concentration noise is up to 40% lower compared to mRNA copy number noise. This indicates that cell growth, an inherent property of living cells, is a main contributor to gene expression variability in terms of absolute copy numbers.

Our results are in line with the results of Padovan-Merhar et al. and Battich et al, which showed that cell volume variability indeed explains a considerable fraction of expression variability of mRNA copy numbers [40, 197]. Padovan et al. observed a similar trend of mRNA concentration homeostasis. In addition, they showed that their data is best explained when the transcription rate is volume-dependent whereas the degradation rate is volume-independent (in terms of mRNA numbers). Their data suggest that the mechanism behind mRNA concentration homeostasis is an increased burst size of mRNA transcription during cellular growth (i.e. DNA dilution). Battich et al. [40] showed that besides the major contribution of cell volume, gene expression levels scale with many other phenotypic variables (e.g. total protein content). This clearly indicates the importance of analyzing gene expression models within their biological context. Knowledge of parameters that contribute to gene expression regulation such as growth, genome context, and epigenetic state enables us to understand how variability drives biological behavior.

7.2 A theoretical framework of cell growth allows the simulation of gene expression in growing cell populations

In chapter 3 we focus on the cell growth processes to study the effects of volume growth on gene expression. We measured single cell growth and gene expression in *E. coli* and *B. subtilis* using fluorescence time-lapse microscopy. We showed that single cell bacterial growth is described by a theoretical framework for populations of cells in balanced growth. We used this theory to construct a simulation method that can simultaneously simulate cell growth and gene expression. We validated the obtained simulations on protein expression data. The developed simulation method has been implemented in StochPy (a python simulation package), which allows simulations and analyses of gene expression networks in growing populations. This chapter provides a starting point in elucidating the impact of cell growth, cell division and DNA replication noise on gene expression. The impact of DNA replication on gene expression noise is known for a simplified cell growth model with fixed interdivision times in combination with a linear Birth-Death model of gene expression [96, 115]. The simulation package we developed enables the integration of more complex regulatory networks. It also allows the assumptions regarding cell growth and replication to be loosened approximating cellular growth more realistically. Padovan-Merhar et al. [197] observed that cells compensate for an increase of DNA after DNA replication in order to maintain mRNA concentration homeostasis. This compensation is proposed to result from DNA or histone modifications, which get redistributed equally to new DNA strands. This dilution of histone modifications during replication is proposed to change the burst frequency of transcription. Our model, although designed to simulate bacterial cell growth, should be able to test whether hypotheses on regulatory mechanisms of gene transcription such as those proposed by Padovan-Merhar et al. [197] can adequately describe gene expression variability in growing cells.

7.3 A chromatin-dependent delayed response of gene deactivation

As discussed in chapter 2, the mean and variability of gene expression are dependent on the local gene context. We measured gene expression of a target gene integrated at three different genomic locations and observed distinct expression profiles dependent on the genomic position. The local epigenetic chromatin state is known to influence gene expression dynamics at different genomic locations [198]. To determine how the chromatin state influences expression dynamics, we determined the transcription repression dynamics of a reporter gene array upon changing the chromatin state of the array in single cells (chapter 4). After removing the transcription activator, we observe that transcription initiation transiently continues. Changing the chromatin state by methyl-CpG binding protein 2 (MeCP2) decreased the time-delay of inactivation from

five to two minutes. This suggests that the local chromatin structure can determine the duration of transiently continuing transcription initiation in the absence of a transcriptional activator. The impact of different chromatin states on transcription regulation in single cells was also shown by Bintu et al. [164]. The authors combined targeted recruitment of chromatin regulators with time-lapse microscopy to determine the timing and duration of chromatin regulator-induced reporter gene repression. Besides the impact on the timing of transcription silencing, they observed that reinitiation of transcription is also largely influenced by chromatin regulators. Bintu et al. [164] conclude that chromatin regulators provide distinct memory regulation of the gene expression state.

7.4 The half-life of DNA damage, which determines the transcription recovery rate, varies depending on UV-dosage

DNA damage as a result of UV-exposure can result in termination of the expression of affected genes [184, 186]. The nucleotide excision repair system is able to detect and repair DNA damages to restore transcription and enables cell survival following UV-exposure [184]. In chapter 5 we showed that the transcription recovery rate after UV-damage is dependent on the UV-exposure. We used single-molecule mRNA counting to measure the effect of UV-induced DNA damage on gene expression. Using the transcription inhibitors DRB and actinomycin D, we showed that transcription inhibition can be measured using single-molecule mRNA FISH. We demonstrated that when a UV-C dose is administered which (theoretically) damages all target genes in a cell population, transcription inhibition was similar to inhibition induced by chemical inhibitors of transcription. Our single-molecule mRNA-FISH method allows us to measure the impact of UV-induced DNA damage on specific genes with high sensitivity. We observed that transcription of short genes is relatively unaffected by UV-exposure in contrast to larger genes. This difference in transcription inhibition upon UV-exposure may well be due to the increased likelihood to acquire DNA damages in larger genes. It has been shown that nucleotide excision repair acts through distinct mechanisms at different UV-doses, i.e at low UV-doses ($< 4\ J/m^2$) only transcription-coupled repair takes place, whereas at higher UV-doses global repair is also involved [187]. In line with those results, we measured two distinct half-lives of DNA-damages at 3 J/m^2 and 5 J/m^2 UV-C-exposure. This supports the notion that distinct DNA damage recognition systems are active at different UV-dosage.

7.5 Single cell analysis reveals that subpopulations of breast cancer cells may cause treatment resistance

Approximately 70% of human breast cancers are estrogen receptor alpha (ERα) positive [190]. In this breast cancer subtype, cell proliferation is stimulated by the binding of estrogen to ERα, which activates transcription of ER target genes. One of the most effective treatments of ERα-positive breast cancers targets ER functioning, for example by blocking estrogen production through the inhibition of aromatase [191, 192]. However, recurrence is observed in up to 30-40% of patients [199–201]. It is currently unknown how resistance development to the treatment functions mechanistically. It has been suggested that somatic mutations involved in ERα-activation may be involved in metastatic progression [195]. Alternatively, counteracting regulatory pathways are altered through epigenetic regulation [194]. It is expected that changes in the expression level of CYP19A1, encoding aromatase, induces resistance and stimulates metastatic progression. We demonstrate that subpopulations of ERα-positive breast cancer cells overexpress aromatase (chapter 6). This could be due to amplification of the CYP19A1 gene locus, especially since it was observed that in long-term estrogen deprived cells multiple DNA copies of CYP19A1 were observed [193]. Alternatively, aromatase overexpression might be related to gene expression variability. Our results demonstrate how treatment resistance may be the result of only a single or a few cells with an altered expression status.

Overall, we conclude that various parameters contribute to gene expression cell-to-cell variability. We demonstrated that mRNA copy numbers scale proportionally with cell volume and propose that functional variation in gene expression is best captured by concentration variability. Based on these findings we developed a simulation tool to analyze gene expression in growing cells. Next, we used single cell transcription measurements in defined experimental systems to determine how cells react to an altered epigenetic composition or to stress-inducing exposure. With these experimental systems we showed that changes in the epigenetic state accelerate the response of active genes to transcription repressing signals, we determined the transcription recovery rate after UV-damage-induced transcription stalling, and we showed that transcriptional upregulation in a subpopulation of breast cancer cells may induce drug resistance. Based on our findings in this thesis we can conclude there are many factors contributing to noise, i.e. cell growth, cell volume, genomic context, epigenetic composition and stress. In the future it might be shown how all these factors of variability integrate and whether these fluctuations are also transferred to the functional level and drive biological behaviour.