7 SUMMARY

The overall aim of this study was to gain global insights into the role of histone modifications and their potential interactions with four key cardiac transcription factors in the context of heart development and congenital heart diseases. While histone modifications influence the compaction of chromatin and consequently the accessibility of DNA, transcription factors (TFs) are responsible for a fine tuning of expression. On both levels of regulation hundreds of factors are known to act, and many are probably as yet undiscovered. Little is known about the mechanisms by which histone modifications or transcription factors function and even less is known about the interplay between them. To gain insight into the involved processes four examples from each of these two regulatory levels were chosen, i.e. four histone modifications and four transcription factors. Because congenital heart disease is the most common birth defect in humans, understanding of processes governing heart development are of particular relevance. Consequently, heart and skeletal muscle cells were chosen as model systems in this investigation.

According to the *histone code hypothesis* different combinations of modifications might lead to distinct read-outs. This hypothesis was tested by investigating four activating histone modifications in three different cell types. Their combinatorial occurrence was determined by chromatin immunoprecipitation followed by detection on custom arrays representing the 12,625 transcription start sites of 8,585 murine genes. This information was combined with expression array data of the same genes. The array designs, the software package for the analysis as well as the raw data and a results database were made available on the internet. The presented data set demonstrated that the average transcript levels associated with combinations of modifications are not simply related to those associated with individual modifications, supporting that they indeed form a *code*: Different combinations of modifications are associated with significantly different transcript levels and the levels of these combinations are not additively related to the levels associated with the individual modifications.

Investigating the dynamics of the same modifications within one cell line during differentiation, the appearance of modifications were, by themselves, rarely associated with higher transcript levels. This is consistent with the view that these marks are a prerequisite, but not a sufficient driving force or a necessary result of transcription. This observation suggests that histone modifications may act as signaling marks for the recruitment of TFs.

The regulatory properties of four transcription factors known to be essential for heart development were investigated in cardiomyocytes. It could be shown that Gata4, Mef2a,

Nkx2.5, and Srf form a regulatory subnetwork in which they not only regulate each others expression but also have a high number of co-regulated target genes. This report presents a comprehensive catalogue of the genes regulated by these important players in heart development. A high number of these targets were again found to be TFs, suggesting that Gata4, Mef2a, Nkx2.5, and Srf can be placed at the top of several regulatory cascades. The refinement of existing and the discovery of novel binding motifs for these TFs provide tools for the future prediction of direct target genes.

By combining this data with the levels of the target genes in HL-1 cells where the respective TF proteins levels were reduced through RNA interference, the functionality of the TF binding could be assessed. Each of the TFs was found to be an activator for the majority of its direct targets. In case of Gata4 and Srf this activating potential was significantly enhanced at binding sites showing acetylation of histone 3. This might be a consequence of their interaction with the histone acetyl transferase p300. Overall, approximately 80% of transcription factor biding sites (TFBSs) were found to occur at histone modified sites, a further indication that these may indeed function as signaling marks for the recruitment of TFs. However, as the different histone modifications frequently occur together further studies will be necessary to characterize the precise mode of interaction.

The investigative power of this approach was exemplified for Tbx20. Previously, the mechanisms governing the expression of this essential cardiac T-box factor were unknown; now it could be demonstrated, that the promoter and first intron are marked by the specific histone modifications H3ac, H4ac, H3K4me2 and H3K4me3 in heart, but not in skeletal muscle cells. At the positions where a maximum enrichment of these modifications was measured three binding sites of Gata4, Mef2a, Nkx2.5, and Srf were identified and each contained the TF binding motif. Knockdown experiments using RNA interference confirmed that Gata4, Mef2a, Nkx2.5, and Srf each activate the expression of *Tbx20*.