SUMMARIES

Summary

DNA contains all genetic information which makes people unique individuals. Each cell in the human body contains 23 chromosomes which together contain about 40,000 genes. All these genes encode for different proteins which are important for the biological processes in the body. Many genes are expressed in all the cells of the human body, whereas a smaller number of genes is expressed only in specific cell types. One such cell type is the red blood cell or erythrocyte. The erythrocyte originates from a so called pluripotent cell, the HSC. This cell undergoes several steps of differentiation in a process termed erythropoiesis, with the resulting erythrocyte being committed to the production and accumulation of haemoglobin, the oxygen carrier in the blood. Haemoglobin contains two α-globin chains and two β-globin chains, which are encoded for independently by the α-globin genecluster and the β-globin genecluster.

The human β-globin locus is composed of five developmentally regulated genes, which are arranged in the order of developmental expression, and a LCR, containing five DNase I hypersensitive sites, upstream of the genes, which plays an important role in de regulation of the expression of the globin genes and is required for high levels of gene expression.

The work presented in this thesis has been primarily concerned with the regulatory elements present in the human β-globin locus, but also an important part of the work has addressed the basis of the transcriptional activation of the murine globin gene loci. We have shown that the activation of the murine α- and β-globin genes takes place in a stochastic fashion and we have provided evidence that the decision to activate transcription is made at a step prior to the actual transcriptional activation, probably at the level of chromatin organisation. Furthermore, these studies have shown that the activation of the α-locus takes place prior to that of the β-locus during erythroid differentiation.

The studies on the regulatory elements of the locus have concentrated on the hypersensitive sites of the LCR and the Enh and F putative silencers downstream of the $^{A}\gamma$-globin gene. We have deleted both HS2 and HS3 and the Enh and F elements via homologous recombination in a PAC vector, which contains the entire human β-globin locus as a 180 kb insert. Our studies show that each element has an individual role, but also complex interactions between the different elements are required for globin gene activation and developmental regulation.

HS2 and HS3 were deleted to investigate the role of LCR function in chromatin organisation and gene activation. We show that the deletion of HS2 has little effect on the LCR chromatin opening properties, since in only one out of four transgenic lines with this site deleted PEV is observed. We do, however, observe a severe effect on ε-globin expression, indicating that HS2 may play a role in the ε-globin gene activation in the embryo. The HS3 deletion shows a severe impairment of gene expression, which increases with development. This appeared to be caused by PEV expression of the transgenic locus, indicating that in contrast to HS2, HS3 does seem to play a role in the chromatin opening activity of the LCR. A direct effect on a specific globin gene was not observed with this deletion.

The Enh and F elements were deleted in order to assess their role in the developmental regulation of γ-globin gene expression. These elements reside in the $^{A}\gamma$-δ intergenic region, which has been suggested to play a role in the γ→β switch and the regulation of γ gene silencing in the adult, since deletions within this region are associated with the hereditary persistence of foetal haemoglobin (HPFH). The deletion of the Enh and F elements, however, did not affect the silencing of the γ genes in the adult, but instead resulted in an increase in the levels of ε- and γ-globin expression in the embryonic stage.

In conclusion, the studies presented here show:

1. that there is evidence for a stochastic basis for activation of globin gene expression;
2. that the LCR does function as a holocomplex, but also that the individual hypersensitive sites act differently on each globin gene;
3. that the regulation of the individual globin genes is very complex and probably involves the interaction of various combinations of elements during different stages of development.