

7 Summary

Disease-causing mutations are commonly located within the open reading frames of mRNAs and are likely to affect the protein structure and function. However, several hereditary diseases do not result from mutations which exert these obvious effects, but have been shown to influence various steps of mRNA metabolism. In this study two examples of disease-producing mutations have been used to investigate post-transcriptional mechanisms affecting gene expression.

In the first part of this thesis, the molecular mechanism underlying the prothrombin 20210 G→A mutation has been elucidated. This mutation represents a very common genetic risk factor for the occurrence of thromboses and affects 1-2% of the European population. The G→A transition is located at the last nucleotide of the 3'UTR of the mRNA and causes elevated prothrombin plasma levels, which are thought to play an important role in the pathogenesis of the disease. Here I show, that the normal prothrombin 3'end processing signal is inefficiently recognised and that the G→A transition is a gain of function mutation causing an increase of processing site usage. This results in an elevation of correctly 3'end processed mRNA accumulation in the cytoplasm and increased protein synthesis. Enhanced mRNA 3'end formation efficiency emerges as a novel molecular principle causing a hereditary disease and explains the role of the prothrombin 20210 G→A mutation in the pathogenesis of thrombosis. Furthermore this work also represents a striking example of how a quantitatively minor aberration of mRNA processing can predispose to a common and serious disease.

A highly conserved mRNA quality-control mechanism referred to as nonsense-mediated mRNA decay has been investigated in the second part of this thesis. This mechanism, which leads to the rapid degradation of nonsense-mutated mRNA, plays an important role as a modifying factor in a number of hereditary diseases. The important role of the NMD-pathway is exemplified by mutations in the β -globin gene, leading to β -thalassemia. The β -globin gene was also used in this study to analyse functional characteristics of the NMD. Experiments

addressing the role of the poly(A) tail for the NMD-pathway showed, that human NMD occurs poly(A) independent. Furthermore, an experimental system has been developed to identify and characterise protein factors involved in human NMD. A number of deletion mutants of the human NMD-factor hUpf3b were created and tested for their ability to elicit NMD in this system. This approach showed that a sequence, subsequently identified as Y14 interaction site, played an essential role for the NMD-activation by hUpf3b. This indicated an NMD function of Y14. In further experiments, direct analysis of Y14 resulted in a dramatic NMD-activation. Together, these experiments define an important role of Y14 in the human NMD-pathway.

In summary, these results provide an important contribution to our general understanding of effects of disease-causing mutations on various steps of the mRNA-metabolism. Furthermore they will have a remarkable impact on future investigations of the molecular pathogenesis of hereditary diseases.