5b Summary

During the work for this thesis several aspects of the analysis of complex genomes were explored. Firstly different hybridisation based approaches for genetic mapping were tested in the genomes of zebrafish and medakafish. Such approaches should allow efficient mapping of new markers to a high resolution. IRS-PCR is the simplest possibility to generate markers that can be mapped by hybridisation. It takes advantage of interspersed repetitive sequences in the genome to amplify a set of unique DNAs between two of such elements. The PCR-products were cloned and around 1000 individual clones of a marker library containing 16 thousand clones were tested for polymorphism on small southern blots of complex IRS-PCR product of different zebrafisch strains. Those markers identified polymorphic were subsequently hybridised on mapping filters onto which IRS-PCR-products of individuals of four different mapping crosses were spotted. Two major difficulties arose with this approach: Because of the redundancy of the library clones were tested repeatedly. This problem could be overcome by analysing the whole marker library by oligo-fingerprinting and grouping the clones with similar fingerprints into clusters. Representatives of different clusters were then used as hybridisation probes and sequenced. The major problem, however, was the high degree of variability within the zebrafish strains. Because of this less than 30 % of the markers that were identified polymorphic on Southern blots were informative on a reference cross and could be placed on a map. Despite of the encountered problems around 80 markers could be scored on one of the reference crosses, and 50 of those showed linkage to at least one further clone.

The same strategy was also tested for the Medakafish, however with a combination of primers, specific for three different repetitive elements. From the 3800 clone medaka library hundred clones were tested on Southern blots. The rate of polymorphic markers was almost 30% and compared to the zebra fish more than twice as high. Moreover 50 % of the makers previously identified polymorphic were informative on an F_2-intercross of different strains. This indicates a much higher genetic distance between the different strains than in the zebrafish as well as a higher degree of homozygocity within the strains. In order to generate further markers that could simultaneously be linked to genomic clones a modified AFLP-technique was developed. After differential hybridisation of two amplicons generated from different strains on a cosmid library 80 clones with a differential hybridisation pattern could be identified. It was possible to isolate a probe from 20 % of the cosmids, and more than 50% could be mapped on an F_2-intercross.

A further aspect of the analysis of complex genomes is the comparative sequence analysis. By comparison of genomic sequences of different species it is possible to get insight into gemone evolution and organisation. Within this work a 40 kb clone of an amphioxus cosmid-library was sequenced, analysed with different exon prediction programs and the deduced protein sequences were compared with two public and one in house sequence database. This led to the identification of a gene of the aldo-keto reductase family as well as further exons which gave a significant database match to known genes. Furthermore it could be demonstrated that genomic sequence analysis and EST projects complement each other well.