7 Summary

In order to gain a deeper understanding of bone development and regeneration it will be necessary to learn more about the genes involved in these processes by clinical and basic-science research. Evolutionary biology can also provide us with valuable hints about skeletal biology.

In the present work cDNA libraries from different stages of sheep fracture callus tissue were constructed from which over 47,000 expressed sequence tags (ESTs) were sequenced. The EST distribution for genes in the different cDNA libraries was analyzed, and quantitative RT-PCR analysis was additionally performed for 87 genes with a potential function during fracture healing. Among these were 53 genes, for which a function in skeletal devlopment or in fracture repair had not been previously reported. Besides the identification of novel candidate genes for fracture healing, the EST sequences are an important resource for research using the sheep as model organism.

In a developmental approach, expression profiles of E14.5 *Runx2* wildtype and knockout mouse humeri were compared in order to find genes with relevance for skeletogenesis. *Runx2* encodes a transcription factor that is essential for skeletogenesis. In this screen, 71 transcripts were found to be differentially expressed and were confirmed by real-time PCR. For 31 of these genes, a role in skeletogenesis has not yet been described. After determination of the expression patterns of the candidate genes, an evolutionary biology approach was used to identify potential direct Runx2 target genes. Promoter regions of candidate genes were screened for Runx2 binding sites that are conserved between different species. Using this approach five novel potential Runx2 target genes were identified.

Furthermore the analysis of *Runt* gene family evolution led to novel insights into the molecular mechanisms of skeletogenesis. Cloning and phylogenetic analysis of three *Runt* homologous genes of the lesser spotted dogfish *Scyliorhinus canicula* showed that the dogfish *Runt* genes are orthologs of the mammalian *Runx1-3* genes. Expression analysis of the *Runt* homologous genes of dogfish, hagfish and amphioxus as well as the amphioxus *Sox9* indicates, that these genes have an evolutionarily conserved function in development of the skeleton and its precursor structures. It could be shown, that this molecular machinery, which is essential for skeletogenesis, is also present in cephalochordates and not only in vertebrates and is thus approximately 100 million years older than previously thought.