Summary

The *Hox* genes form a subset of the homeobox containing genes. The homeobox encodes a DNA binding motif, called the homeodomain. In most animal species the *Hox* genes are organised in one or more clusters. The number of genes present in a cluster varies between animal species; the number of clusters in each species also varies. The Hox clusters are thought to have arisen by tandem duplication of a single gene, followed, in vertebrates, by duplication of the cluster itself. As a consequence, *Hox* genes occupying the same relative position along the 5' to 3' chromosomal coordinate, named paralogous genes, share more similarities in sequence, expression pattern and function than do adjacent *Hox* genes on the same chromosome. One of the conserved features of the *Hox* genes is that of spatial colinearity of *Hox* expression along the AP axis and other embryonic axes. This means that paralogs located in the most 3' position of a cluster are expressed at a more anterior position than genes located at more 5' clustral locations. Vertebrate embryos also display a second form of colinearity, temporal colinearity, whereby genes located more 3' in the cluster are expressed earlier than genes located more 5' in the cluster.

It has been firmly established that *Hox* expression boundaries along the AP and other embryonic axes are correlated with structural identities. Generating correct *Hox* expression patterns is thus clearly essential for correct AP axis patterning. The aim of investigations presented in this thesis has been to gain more insight into the processes controlling expression and function of *Hox* genes during anteroposterior patterning.

Recent work in our research group has shown that a temporally colinear expression sequence of *Hox* genes is already present in the marginal zone mesoderm of *Xenopus* gastrulae. Most, if not all, previous work concerning *Hox* colinearity has been focused on colinear *Hox* gene expression in the neurectoderm. In chapter two, we present data to show that *Xwnt8* is directly upstream of *Hoxd1* in marginal zone mesoderm. This is the first example of an initiator of expression of a 3' *Hox* gene in a vertebrate.

An upstream regulator of *Hox* gene expression in the neurectoderm of vertebrates is retinoic acid (RA) (and or its derivatives) Retinoids can act via the nuclear receptors of the RAR and RXR family. These receptors form heterodimers and bind Retinoic Acid Response Elements (RAREs) in the promoters of target genes. RAREs have been found in the regulatory sequences of a number of labial- and deformed group *Hox* genes. We took advantage of the availability of the genomic sequences of all four *Hox*
clusters of mouse and human, to search for conserved RAREs in the Hox clusters, and the results are reported in chapter three. Most Hox proteins contain a second conserved domain, in addition to the homeodomain, the hexapeptide. This domain is needed for Hox/PBC member interaction, and this interaction leads to increased binding specificity and affinity. Sequence analysis of Hox proteins demonstrates interspecies sequence conservation among paralog group members in the sequences flanking the hexapeptide, which is reported in chapter four. Nuclear localisation of PBC family members is controlled by competing nuclear import and export signals. Interaction of Meis family members with PBC members shields the nuclear export signal of PBC proteins, resulting in a net influx into the nucleus, modifying the activity of Hox proteins present. In zebrafish hindbrain development, a synergistic relation between Hoxb1, Pbx4, and Meis3 has been shown, and was argued to directly induce the expression of Hoxb1. Since recent discoveries have shown that Hox genes are expressed in a colinear sequence in marginal zone mesoderm it was investigated whether a Xenopus Meis homolog, XMeis3, cooperates with Hox function during gastrula stages. In chapter five, we report that XMeis3 is necessary for mesodermal and ectodermal Hox expression, and the progression of gastrulation.

In chapter six, conservation of hexapeptide-flanking sequences of Pdx1 and Cdx proteins, present in a wide range of species, resembling the conservation found in Hox group 1 through 8 proteins (Chapter four) is reported. More generally the presence of a hexapeptide sequences and conservation of flanking sequences in all of the members of the Antp-class of homeodomain proteins was investigated, and found to be widely distributed, in addition, hexapeptide flanking sequences conservation was found. Identifying the downstream targets of Hox genes is necessary if their function in development is to be understood in molecular terms. XRap1 joins a very short list as to date very few Hox targets have been identified. In chapter seven, the small GTPase XRap1 is reported as a direct target of Hoxb4 regulation. Hoxb4 represses XRap1 expression in a manner that is independent of protein translation, and may bind to two putative HOXB4 protein-binding sites located at the 3’ end of the Rap1 gene in order to mediate this inhibition.