

5 SUMMARY

The *HOX* genes encode an evolutionary conserved family of transcription factors playing an important role in embryonic development. In particular, the posterior *HOXD* genes are involved in limb patterning in higher vertebrates. Different *HOXD* mutations are associated with limb anomalies in humans and in mice. One of them, synpolydactyly (SPD), a severe dominant disorder characterised by digit duplications and webbing, has been connected to various mutations within the *HOXD13* gene.

In this study the chromosome translocation t(2;10)(q31.1;q26.3) present in a male patient with mental retardation and SPD was investigated. The breakpoints were mapped and cloned using cytogenetic and molecular approaches. The results indicated that on chromosome 10, *MGMT*, a gene coding for a DNA-repair enzyme has been disrupted. However, up to now there is no link between this gene and limb development, suggesting that *MGMT* is not responsible for the skeletal abnormalities present in the patient. On chromosome 2, the breakpoint occurred approximately 390 kb centromeric to the *HOXD* complex and did not truncate any known gene. However, it has been recently shown that changes in the global chromosomal environment might lead to misregulation of gene expression by so-called position effects. Therefore, it is likely that the translocation affected the precise regulation of *HOXD* expression resulting in their loss of function and leading to limb abnormalities in the patient.

Studies on molecular bases of limb development and pathogenesis performed during the last years led to identification of many pathways and mechanisms responsible for these processes. However, many questions still need to be answered. To find new players involved in distal limb patterning, I have searched for Hoxd13 interaction partners using the yeast two-hybrid technique. Several candidates were identified and further tested in yeast and mammalian systems. One of them, Peg10 has been shown to co-localise with wildtype and mutant Hoxd13 proteins in COS1 cells. Moreover, binding between these proteins was confirmed in the coimmunoprecipitation assays. Whole mount *in situ* hybridisation experiments indicated that *Peg10* is expressed in distal limb buds during mouse embryogenesis. At the early stages of limb development (E10.5 and E11.5) the *Peg10* expression domain overlaps with that of *Hoxd13*, suggesting that both proteins might interact *in vivo*. The results presented in this study together with literature data suggest that Peg10 could modulate Hoxd13 function and

that both interacting proteins might co-operate in order to regulate transcription of various target genes. However, additional experiments will be necessary to confirm this hypothesis and to explain in detail the role of Hoxd13/Peg10 complexes *in vivo*. Moreover, it would be interesting to further analyse two other potential Hoxd13 binding partners, Limd1 and Cnot3. Binding assays and functional studies could give us new data valuable for better understanding limb patterning processes.