Study of the v-Myb oncoprotein variable region

The aim of this thesis was to analyze the significance of the variable region of oncoprotein v-Myb.

The v-*myb* oncogene originated in the chick through a natural recombination of the MAV1 retroviral genome with the c-*myb* gene, which is an important transcriptional regulator involved in hematopoiesis and other important processes in different types of vertebrate cells. In comparison with the c-*myb* coding sequence, the retroviral v-*myb* is truncated on N- and C-terminals and includes 11 point mutations. Due to both these changes and the retroviral promoter which drives its expresion, the v-Myb oncoprotein became the inducer of acute myeloid leukemia in chicks.

The variable region of v-Myb lies in between the N-terminal DNA binding and the central transactivating domains. This region is the least evolutionarily conserved part of the protein. No published data are available about its potential function.

In this work the biological significance of the variable region was studied by means of specific deletions and sequence swaps.

Two deletions (ΔPstI and ΔNaeI) and two swaps (chicken v-*myb* to mouse and *Xenopus* c-*myb*) were prepared. Recombinant DNAs were cloned into MAV1-based retroviral vector and transfected into chick embryo fibroblasts (CEFs) in tissue culture. The recombinant viruses produced by CEFs were used for infection of chicken bone marrow cells in vitro. Retroviruses with swaps were also used for *in vivo* experiments. Transformation of myeloid cells *in vitro* and *in vivo* was monitored by smears of cultured cells or peripheral blood cells, respectively. Levels of recombinant proteins in transfected and transformed cells were detected by Western blotting and their subcellular localization was examined by indirect immunofluorescence.

It was observed that manipulations with the variable region do not significantly affect transforming capacity of the oncoprotein but rather result in changes in its subcellular localization. Based on these observations it is hypothesized that the variable region is involved in the mechanism of nuclear transport of the oncoprotein.

Another goal was to identify the so far unknown alternative c-*myb* exon 9A in *Xenopus*. This sequence was cloned and sequenced.