5. Summary

The antiapoptotic Bcl-x<sub>L</sub> protein plays a key role in the control of apoptosis and in the pathogenesis of a variety of malignant diseases. The influence of the 5’-noncoding region of the <em>bcl-x</em> gene and the influence of the 5’–nontranslated region of the <em>bcl-x</em> mRNA on the expression of the <em>bcl–x</em> gene has been investigated in this work.

Previously unknown parts of the human <em>bcl-x</em> gene were sequenced and the sequence of the hitherto unidentified complete human <em>bcl-x</em> mRNA-sequence was determined. Identification and detection of mono- and bicistronic spliceforms of the <em>bcl-x</em> mRNA in several human cell lines and mapping of different transcription start sites in the 5’–NCR of the human <em>bcl-x</em> gene helped identify different mechanisms for the control of <em>bcl-x</em> gene expression.

The position of several transcription start-sites and the location of the major promoter activity in the 5’-noncoding region of the human <em>bcl-x</em> gene support the hypothesis that the selection of certain transcription start sites and transcriptional activity are controlled by downstream promoter elements.

Functional analysis revealed that the 5’-nontranslated region of the human <em>bcl-x</em> mRNA contains an internal ribosome entry site (IRES) which facilitates the translation of this particular mRNA by a CAP-independent mechanism. These results lead to the conclusion that the expression of the human <em>bcl–x</em> gene is significantly controlled at the level of translation. This constitutes the first example of an apoptosis control gene regulated by IRES-mediated initiation of translation.

Transient expression of antisense and anti-antisense RNA which correspond to the 5’–nontranslated region of different <em>bcl-x</em> mRNA forms enabled the identification of the preferentially translated <em>bcl-x</em> mRNA form. In addition, these experiments show for the first time that CAP-independent translation of the <em>bcl-x</em> mRNA can be competitively inhibited by the expression of truncated RNAs containing only the translational control sequences.

A cellular <em>bcl-x</em> antisense RNA that shows no influence on the <em>bcl-x</em> gene expression was identified and detected in several human cell-lines. The low concentration of this antisense RNA and the existence of two bicistronic <em>bcl-x</em> mRNAs support the hy-
pothesis that the cellular bcl-x antisense mRNA may have a catalytic function in trans-splicing.

Finally, determining the equilibrium concentration of a protein in a certain tissue or cell type from the total concentration of its mRNA variants is not acceptable if the mRNA variants are translated differentially.