Histol Histopathol (2001) 16: 595-601

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Cellular and Molecular Biology

Review

Transcriptional regulation of the *bcl-x* gene encoding the anti-apoptotic Bcl-x_L protein by Ets, Rel/NFκB, STAT and AP1 transcription factor families

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Summary. Transcription factors play an essential role in determining the fate of a cell by affecting the expression of target genes involved in proliferation, in differentiation and in programmed cell death. Under certain conditions, some of these factors are capable of deregulating expression of genes involved in the cell cycle and/or in programmed cell death resulting in uncontrolled proliferation of the cell. The focus of this review is on the transcriptional regulation of the *bcl-x* gene encoding the anti-apoptotic Bcl-x_L protein. Since 1999, several papers have implicated members of the Ets, Rel/NFκB, STAT and AP-1 families as transcription factors regulating *bcl-x* expression. A specific emphasis of these different transcription factor families on *bcl-x* regulation in hematopoietic cells is discussed.

Key words: bcl-x, Promoter, Regulation, Transcription factors, Review

Introduction

Programmed cell death by apoptosis is a process essential for normal development and maintenance of cell homeostasis in organisms. Proteins of the bcl-2 family have been identified as initiators or inhibitors of apoptosis. The balance in the levels of expression of pro-apoptotic versus anti-apoptotic members is crucial in determining the fate of a cell: a more abundant expression of an anti-apoptotic member will permit a cell to survive whereas a higher expression of a pro-apoptotic member will induce apoptosis upon activation by a variety of apoptotic signals.

Anti-apoptotic members include Bcl-2, Bcl-x_L (Boise et al., 1993), Bcl-w (Gibson et al., 1996), A1 (Lin et al., 1996) and Mcl-1 (Kozopas et al., 1993). Bcl-2, the first anti-apoptotic member identified, and the closely related Bcl-x_L are probably the best characterized. Their genomic structures are similar and are believed to have arisen from a common ancestral gene or by gene duplication (Grillot et al., 1997). By maintaining the integrity of mitochondria, Bcl-2 and Bcl-$_{xL}$ inhibit cleavage-mediated activation of the caspase cascade which functions as the ultimate downstream effector pathway of the suicide program degrading proteins necessary for nuclear and cell integrity. Until recently, little was known about how these genes are regulated. In this review, we will focus on the transcriptional regulation of one member of the anti-apoptotic family, Bcl-x_L.

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Bcl-x

The *bcl-x* gene encodes several alternatively spliced messenger RNAs including *bcl-x_L*, *bcl-x_s*, bclΔTM and *bcl-ß* (Shiraiwa et al., 1996; Grillot et al., 1997). Bcl-x_L suppresses whereas Bcl-x_s induces apoptosis (Boise et al., 1993; Fang et al., 1994; Gonzalez-Garcia et al., 1994). The functions of the other two transcripts have not yet been well documented. Bcl-x_L is believed to be the key anti-apoptotic protein expressed in hematopoietic lineages including myeloid precursors and macrophages (Lotem and Sachs, 1995; Chatterjee et al., 1997; Packham et al., 1998; Sevilla et al., 1999), lymphoid cells of the pre-B lineage (Fang et al., 1994; Grillot et al., 1996), and double positive CD4+/CD8+ T cells (Grillot et al., 1995; Ma et al., 1995). Bcl-x_L expression is activated in B cells of the germinal center and in mature resting B and T lymphocytes after activation with mitogens and costimulatory signals (Broome et al., 1995; Grillot et al., 1996). Interestingly, pro-apoptotic Bcl-x_s is not detected in murine T or myeloid cells (Gonzalez-Garcia et al., 1994; Packham et al., 1998; Sevilla et al., 1999). The importance of Bcl-x_L in hematopoiesis has been confirmed by *bcl-x* gene disruption studies in mice which die at E12-13 of embryonic development due to massive neuronal and hematopoietic progenitor apoptosis (Motoyama et al.,

1995). Bcl-x_L expression has been shown to be activated by retroviral insertions in murine myeloid and T cell hematopoietic malignancies demonstrating that the expression of Bcl-x_L can also be altered in cancer (Packham et al., 1998).

The *bcl-x_L* transcript is upregulated in hematopoietic cells upon a variety of stimuli. Among these are stimuli including interleukin-2 (IL-2), IL-3, IL-6, granulocyte macrophage colony stimulating factor (GM-CSF), colony stimulating factor (CSF-1), leukemia inhibitory factor (LIF), and erythropoietin (Epo) which induce the survival and/or proliferation of hematopoietic cells. *bcl-x* has been shown to be an immediate early gene in Epo-treated HCD57 erythroleukemia cells (Socolovsky et al., 1999) whereas in myeloid cells, *bcl-x* was shown to be a delayed early gene whose transcriptional activation is dependent on de novo protein synthesis after CSF-1 (Sevilla et al., 1999) or IL-3 (Packham et al., 1998) stimulation. In addition to the bcl-x_L transcript having a relatively short half life (Sevilla et al., 1999), the expression of the Bcl-x_L protein parallels that of the bcl-x_L transcript (Packham et al., 1998). It was therefore important to clone and characterize the *bcl-x* promoter to gain insight as to how *bcl-x* is regulated at the transcriptional level upon these different cytokine or growth factor signals.

The human and murine *bcl-x* promoters have been isolated and two distinct regions of the mouse *bcl-x* gene have been shown to have promoter activity (Grillot et al., 1997). The first promoter region of 57 bp is found immediately upstream of the first coding exon (exon II) and the second promoter region is upstream of the first non-coding exon (exon I). Major transcription initiation sites have been mapped to both regions. The differences in promoter usage may be attributed to a specific cell type or to the status of differentiation of the cell, although this remains to be determined. Sequence comparisons between the human and mouse promoters permitted the identification of highly conserved consensus binding sites for different transcription families (Grillot et al., 1997). The objective of this report is to review the different families of transcription factors which have been shown to activate the *bcl-x* promoter.

Ets

The Ets family of transcription factors consists of approximately 30 members all possessing a conserved DNA binding domain of about 85 amino acids known as the "E" or "Ets" domain [(Boulukos et al., 1989); reviewed in (Ghysdael and Boureux, 1997)]. Ets1, the progenitor to v-Ets found in the E26 retrovirus, and Ets2 are 97% conserved in the Ets domain (Boulukos et al., 1988), whereas PU.1/Spi.1 is highly divergent in this domain [37% identity; (Wasylyk et al., 1993)]. These differences in sequence identity of the Ets domain allow Ets1, Ets2 and PU.1/Spi.1 to bind to common as well as distinct optimal DNA target sequences known as Ets binding sites (EBS).

In addition to their roles in proliferative and differentiation pathways, several studies suggested that *ets* family members might affect the survival of cells. Apoptosis was observed in *ets*1-deficient T cells from chimeric mice generated by *ets*1 gene disruption in embryonic stem cells with RAG2-/- blastocysts using the recombination activating gene complementation assay (Bories et al., 1995; Muthusamy et al., 1995). In other studies, Jin et al., reported that primary peritoneal macrophages obtained from "dominant negative" Δ*ets*2 transgenic animals died rapidly by apoptosis when depleted of CSF-1 (Jin et al., 1995), a factor necessary for the growth, differentiation and survival of myeloid cells. Finally, other experiments using PU.1/ Spi.1, showed that PU.1/ Spi.1 cooperated with an activated erythropoietin receptor (EpoR) to inhibit apoptosis in primary erythroblasts (Tran Quang et al., 1997). Taken together, these observations suggested that the role in inhibiting programmmed cell death may be a common function of members of the *ets* family, yet the mechanism(s) of this inhibition have not been elucidated in these studies.

Like Bcl-x_L, PU.1/Spi.1 is expressed in hematopoietic precursors and in mature macrophages and *pu.1*-/- mice are devoid of mature B cells, neutrophils or macrophages (Scott et al., 1994; McKercher et al., 1996). Ets1 and Ets2 are specifically and reciprocally expressed during precise stages of thymic development (Pognonec et al., 1987, 1988; Bhat et al., 1989, 1990). Bcl-2 and Bcl-x_L are also expressed in a reciprocal manner during T cell development and interestingly, Ets2 and Bcl-x_L are coexpressed in the same population of developing T cells. Coexpression of Ets2 and Bcl-x_L is not limited to double-positive CD4+/CD8+ T cells since both are also coexpressed in primary bone marrow macrophages (BMM) derived from bone marrow progenitor cells and in BMM upon activation of functional competence signals (Sevilla et al., submitted).

Transient expression of Ets2 in human 293 cells upregulates Bcl-x_L protein expression (Sevilla et al., 1999). Constitutive expression of Ets2 in a CSF-1-dependent macrophage cell line (BAC1.2F5) also upregulates Bcl-x_L expression in a constitutive manner and renders these cells resistent to apoptosis when depleted of CSF-1 (Sevilla et al., 1999). The most plausible mechanism of upregulation of Bcl-x_L by Ets2 was by transcriptional activation.

Both the 5' regulatory sequences found upstream of the first noncoding exon of *bcl-x* and the first facultative intron contain potential EBS for Ets transcription factors. Eight EBS are found within a 700 bp fragment of the human promoter upstream of the first non-coding exon and one in the first intron (Fig. 1), while the predicted number of EBS found in a random 700 bp sequence is 1.07. The conservation of the human and mouse promoter regions is over 70% (Grillot et al., 1997). Like the human *bcl-x* promoter, the murine *bcl-x* promoter also contains a high concentration of sites (11 EBS in

700 bp). Ets2, but also Ets1 and more distantly related PU.1, all individually transcriptionally activate the *bcl-x* promoter with similar capacities (Sevilla et al., 1999). Deletions of distal sites show that maximal activity requires the integrity of these sites (Sevilla et al., 1999). Together, these results show that different members of the *ets* family play a role in inhibiting programmmed cell death and at least one mechanism of inhibition by Ets transcription factors is by regulation of expression of the *bcl-x* gene.

Rel/NFκB

Rel/NF-κB transcription factors are homo- and heterodimeric proteins consisting of related subunits. Rel/NF-κB transcription factors rapidly translocate to the nucleus upon growth factor, cytokine, viral or stress stimuli and regulate the expression of many genes important for the function of the immune system (Hatada et al., 2000). Members include p50 (NFκB1), p52 (NFκB2), p65 (RelA), c-Rel, the progenitor to v-Rel of the Rev-T avian retrovirus, and RelB [for review see (MacKichan and Israel, 1997)].

Gene disruption studies in mice have demonstrated that Rel/NFκB transcription factors are critical in regulating genes induced during immune responses [see (Grossmann et al., 1999)]. Several *in vivo* studies suggested that Rel/NFκB played a role in the inhibition of apoptosis since inactivation of RelA/p65 in *relA*-/- mice leads to embryonic death due to massive apoptosis of the liver (Beg et al., 1995). Double knock-out *c-rel*-/-*relA*-/- mice die earlier *in utero* than *relA*-/- from increased apoptosis of the liver, and hematopoietic progenitors recovered from *c-rel*-/-*relA*-/- fetal livers fail to support survival of lethally irradiated mice (Grossmann et al., 1999). These results demonstrate that both c-Rel and RelA have overlapping functions and regulate the survival of committed hematopoietic progenitors.

Many genes implicated in inducing or inhibiting apoptosis contain NFκB-like elements in their promoter regions [see Tables in (Dixon et al., 1997; Chen et al., 1999)]. Four recent studies show that Rel/NFκB is important in the regulation of Bcl-x_L expression in cells. Tax, a regulatory protein encoded by the human T-cell leukemia virus type 1, renders the CTLL-2 mouse T cell line IL-2-independent (Iwanaga et al., 1999). This first study showed that the loss of IL-2 dependence and resistance to apoptosis of these cells correlate with an increase of Bcl-x_L expression induced by Tax through a NFκB pathway (Tsukahara et al., 1999). CD40, a member of the tumor necrosis factor receptor family, induces survival of B cells. In another study, Lee et al., showed that NFκB upregulated Bcl-x_L expression and that this upregulation is required for CD40 survival signals in human Daudi and Ramos B cell lines (Lee et al., 1999). In other studies, using human HT1081 fibrosarcoma cells and HeLa cells expressing inducible Rel proteins, Chen et al., showed that both c-Rel and RelA/p65 increase Bcl-x_L expression (Chen et al., 2000). Finally, macrophages from p50-/- mice have increased Bcl-x_L levels and are more resistent to silica-induced cell death (Chen et al., 1999). Silica was shown to activate p50/p50 NFκB1 homodimers in macrophages obtained from wildtype mice and to induce apoptosis, whereas silica activates p65-containing complexes in macrophages from p50-/- mice (Chen et al., 1999).

Two NFκB-like sites are found in the 700 bp *bcl-x* promoter sequence (Fig. 1). Although p65/RelA, p50/NFkB1, RelB and c-Rel all bind to these NFkB-like elements (Chen et al., 1999, 2000; Lee et al., 1999; Tsukahara et al., 1999), only c-Rel and p65 were shown to transactivate the *bcl-x* promoter in hematopoietic cells (Chen et al., 1999; Lee et al., 1999; Chen et al., 2000).

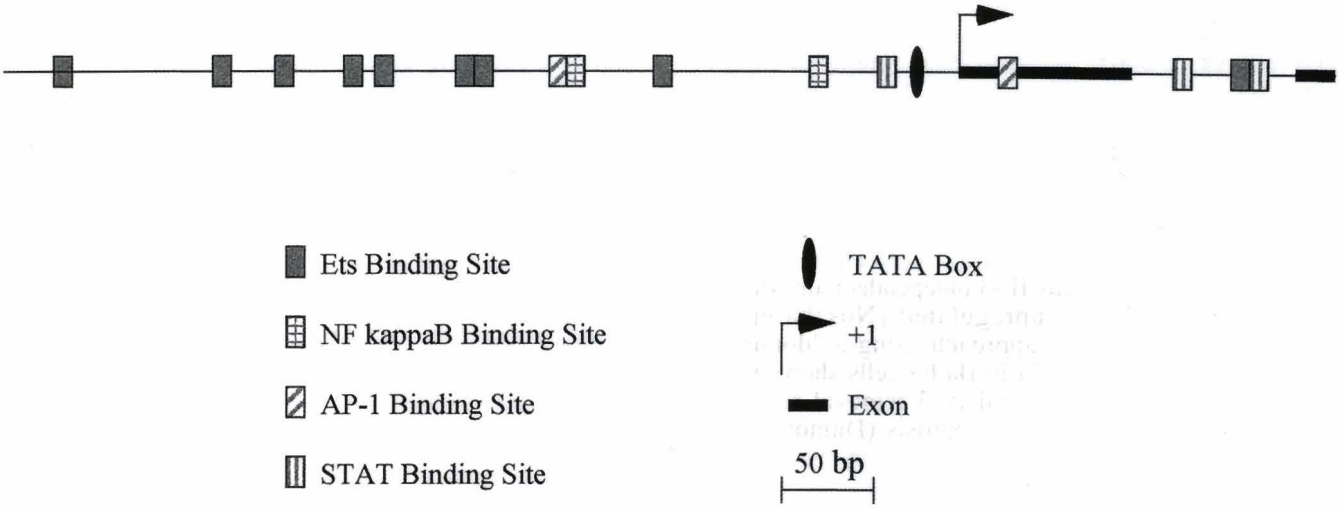

Fig. 1. The structure of the 5' regulatory sequences of the human *bcl-x* gene. Binding sites for Ets, Rel/NF-κB, STAT and AP-1 transcription factor families were determined using the MatInspector program (Quandt et al., 1995). Conserved consensus motifs, TATA box, exons, and a major transcription initiation site (+1) are indicated in the figure.

These results show that Rel/NFκB, another important transcription factor family, plays a role in the regulation of *bcl-x* expression specifically in B and T cells. Rel/NFκB can also function as a negative regulator of Bcl-x_L in double-positive CD4+/CD8+ thymocytes (Hettmann et al., 1999). Therefore, additional experiments are needed to clarify under what conditions Rel/NFκB functions as a positive regulator or as a negative regulator of *bcl-x* expression.

STATs

Signal Transducers and Activators of Transcription (STATs) play key roles in growth factor, cytokine or hormone-mediated intracellular signal transduction. Ligand binding initiates phosphorylation of tyrosine residues and subsequent homo- or heterodimerization of the STAT proteins. This phosphorylation activates STATs to migrate from the cytoplasm into the nucleus, to bind to specific DNA sequences in the promoter regions of certain genes and to activate or repress the transcription of these target genes. Seven members of the STAT family as well as four isoforms lacking their variable transactivation domains have been identified.

It was first reported that Bcl-x_L proteins are regulated by the Jak kinase pathway independent of STATs in hematopoietic cells (Packham et al., 1998). This conclusion was based on experiments using the mouse immortalized myeloid progenitor 32D.3 cell line expressing different EpoR mutants treated with Epo. However, more recent studies from several laboratories indicate that STATs do effect Bcl-x_L expression. Embryos from *stat5a-/- stat5b-/-* double knockout mice show severe defects in fetal liver erythroid progenitors and that these erythroid progenitors undergo increased apoptosis in vitro (Socolovsky et al., 1999). *stat5a-/- stat5b-/-* erythroid progenitors are less responsive to Epo (Socolovsky et al., 1999), a cytokine essential for their growth and survival, and Epo activation of STAT5 in Epo-dependent HCD57 erythroleukemia cells mediates the induction of Bcl-x_L through direct binding of STAT5 to the *bcl-x* promoter (Silva et al., 1999). Only STAT5, and not STAT1 or STAT3, bind to the *bcl-x* promoter in response to Epo in these cells (Silva et al., 1999).

The regulation of *bcl-x* transcription by STAT5 is not limited to erythroid cells. The mouse pro-B Ba/F3 cell line is dependent on IL-3 for its growth and survival. Ba/F3 cells expressing a constitutively active STAT5 mutant rendered these cells IL-3 independent and Bcl-x_L mRNA expression was upregulated (Nosaka et al., 1999). A complementary approach using a "dominant negative" mutant of STAT5a in Ba/F3 cells showed that Bcl-x_L was downregulated and IL-3 removal rendered these cells more sensitive to apoptosis (Dumon et al., 1999). These authors went further to demonstrate that STAT5 transcriptionally activated the *bcl-x* promoter in Ba/F3 cells (Dumon et al., 1999).

Other members of the STAT family may be implicated in the transcriptional regulation of the *bcl-x* gene and this regulation may occur in nonhematopoietic cell types as well. Supporting this notion are studies showing that STAT1, but not STAT3, mediates the expression of the *bcl-x* gene upon LIF treatment of cardiac myoblasts (Fujio et al., 1997).

AP1 complexes

c-Fos and c-Jun, the progenitors of their oncogenic counterparts in murine osteosarcoma viruses FBJ.MuSV and FBR.MuSV, and in a chicken osteosarcoma ASV17, respectively, are immediate early genes which are rapidly activated in response to a wide variety of extracellular stimuli. The *fos* family consists of c-Fos, Fra-1, Fra-2 and FosB and the jun family consists of c-Jun, JunB and JunD. Both Fos and Jun are members of the basic/leucine zipper family. Jun proteins can form homodimers but Fos proteins cannot homodimerize. However, Fos proteins can heterodimerize with Jun proteins and regulate the transcription of target genes whose promoters contain AP1 DNA binding sites. Jun homodimers or Fos/Jun heterodimers are also known as the AP1 complex. Although the expression of Fos and Jun members is often transient, their AP1 activity have been shown to profoundly effect both proliferation and differentiation pathways [for review see (Bannister and Kouzarides, 1997)].

More recently, AP1 activity has been implicated as being potentially important in regulating anti-apoptotic genes (Jacobs-Helber et al., 1998). When c-Jun was present in the AP1 complex of Epo-treated HCD57 erythroid cells, these cells survived. However, Epo withdrawal led to the presence of JunB in the AP1 complex and JunB activity correlated with increased death. A dominant negative mutant of c-Jun inhibited the downregulation of Bcl-x_L expression and rendered HCD57 cells resistant to cell death after Epo depletion (Jacobs-Helber et al., 1998). Characterization of the *bcl-x* promoter revealed the presence of an AP1 site upstream of the first non-coding exon (Fig. 1) and that c-Fos and c-Jun were shown to transactivate the *bcl-x* gene via this AP1 site (Sevilla et al., 1999).

Cooperation of different transcription factor families in regulating *bcl-x* gene expression

The activation of Ets, Rel/NF-κB, STATs and AP-1 transcription factors after stimulation with a variety of extracellular signalling molecules in different hematopoietic lineages clearly correlates with the increased expression of Bcl-x_L. Since members of these different families are rapidly activated upon many of the same stimuli and in the same cell types, several questions remain: is there cooperation between the different classes of transcription factors in *bcl-x* gene regulation and if so, what are these factors and under which conditions do they cooperate? To begin to address these questions, recent studies from our laboratory have investigated the effects on the coexpression of members

of the different transcription factor families on *bcl-x* regulation.

The protein of reference in these studies was Ets2. When STAT5a was activated in the presence of Ets2, STAT5 actually diminished the capacity of Ets2 to transactivate (Sevilla et al., 2001). Either STAT5a was physically interfering with the capacity of Ets2 to bind to EBS, or STAT5a was sequestering an unidentified cofactor from Ets2. A potential cofactor may be a protein of the p300/Creb-binding protein (CBP) family which interacts with both STAT5 (Pfitzner et al., 1998) and Ets2 (Jayaraman et al., 1999) (see below).

Coexpression of c-Fos and c-Jun with Ets2, resulted in an increase in transactivation, but this effect was only additive (Sevilla et al., 2001). Many promoters contain AP-1/Ets binding sites and cooperation between the AP1 complex and Ets is well documented [for review see (Bannister and Kouzarides, 1997) and references therein]. The basic region of c-Jun has been shown to physically interact with the DNA binding domain of several members of the *ets* family including Ets1, Ets2 and PU.1 (Bassuk and Leiden, 1995; Basuyaux et al., 1997). Yet the significance of AP1/Ets activity on the *bcl-x* promoter needs further attention.

Rel/NF-κB and c-Fos and c-Jun are often activated in parallel in the same cell type upon the same stimulus. For example, as seen in this review Rel/NF-κB and c-Fos and c-Jun are all activated in Epo-treated HCD57 cells. It appears that via their leucine zippers, c-Fos/c-Jun interact with the rel homology domain of Rel/NF-κB and stimulate Rel/NF-κB DNA binding (Stein et al., 1993). Rel/NF-κB members have also been shown to collaborate with AP1 and Ets on the GM-CSF promoter in human Jurkat T cells (Thomas et al., 1997). Whether these interactions occur or not on the *bcl-x* promoter after Epo stimulation of erythroid cells or after stimulation of other cell types has not yet been established.

Surprisingly, coexpression of two members of the *ets* family, PU.1 and Ets2, resulted in a clear synergy on the *bcl-x* promoter (Sevilla et al., 2001). This synergy of Ets2 and PU.1 was specific since coexpression of Ets1 with either Ets2 or with PU.1 had no effect on *bcl-x* activity. Since Ets1 is not expressed in macrophages, whereas Ets2 and PU.1 proteins are, the regulation of the *bcl-x* promoter at the molecular level parallels biological expression. Both PU.1 and Ets2 transactivation domains are required for this synergy and the integrity of the *bcl-x* promoter is necessary for maximal synergy between Ets2 and PU.1 since deletions of 2 or more sites greatly reduced the synergistic capacity of these two transcription factors. Interestingly, PU.1 and Ets2 proteins are known to bind to DNA as monomers [reviewed in (Ghysdael and Boureux, 1997)] and do not physically interact (personal observations). Either these proteins do not directly interact or their interactions are weak and/or transient. It is possible that these proteins interact via a third bridging factor. Proteins of the p300/CBP family are coactivators of transcription binding to transcription factors and affecting their transcriptional capacities, and are known to have histone acetyltransferase functions capable of modulating chromatin structure. Recently, p300/CBP have been identified as proteins interacting with PU.1 (Yamamoto et al., 1999) or Ets2 (Jayaraman et al., 1999). Preliminary experiments suggest that PU.1, Ets2 and p300/CBP physically interact and cotransfections of HeLa cells with p300/CBP greatly increased the activities of Ets2 and PU.1 on the *bcl-x* promoter. Therefore p300/CBP appears be a ternary factor involved in Ets2/PU.1 synergistic activity on the *bcl-x* promoter (Zaldumide et al, in prep.).

Conclusions

The Bcl-x_L protein plays an important role in maintaining the integrity of mitochondria thereby inhibiting the release of factors which activate the pro-apoptotic caspase cascade. The level of protein expression parallels that of its mRNA transcript and it is well established that high Bcl-x_L expression correlates with high cell survival in cells subjected to apoptotic signals. *bcl-x* is regulated at the transcriptional level upon activation of cells with a variety of stimuli including survival/growth factors. Recent studies from several laboratories have characterized several transcription factor families involved in regulating the *bcl-x* gene. Members of the Ets, Rel/NF-κB, STAT and AP-1 families of transcription factors have all been implicated in this regulation in hematopoietic cells. Although some of these transcription factor families activate *bcl-x* transcription in an additive or synergistic fashion, further studies will be needed to determine under which circumstances and in what cell types these transcription factors function together to regulate *bcl-x* gene expression.

Acknowledgments. L.S. was supported by EC grant no. ERBFMBICT972684 and by the Foundation pour la Recherche Medicale, and A.Z. by MERNT. Grant support to K.E.B. was provided by Association pour la Recherche contre le Cancer.

References

Bannister A. and Kouzarides T. (1997). Structure/function and oncogenic conversion of Fos and Jun. In: Oncogenes as transcriptional regulators: Retroviral oncogenes. Yaniv M. and Ghysdael J. (eds) Birkhauser. Basel. pp 223-247.

Bassuk A.G. and Leiden J.M. (1995). A direct physical association between ETS and AP-1 transcription factors in normal human T cells. Immunity 3, 223-237.

Basuyaux J.P., Ferreira E., Stehelin D. and Buttice G. (1997). The Ets transcription factors interact with each other and with the c- Fos/c-Jun complex via distinct protein domains in a DNA-dependent and -independent manner. J. Biol. Chem. 272, 26188-26195.

Beg A., Sha W.C., Bronson R.T., Ghosh S. and Baltimore D. (1995). Embryonic lethality and liver degeneration in mice lacking the RelA

component of NF-kappa B. Nature 376, 167-170.

Bhat N.K., Komschlies K.L., Fujiwara S., Fisher R.J., Mathieson B.J., Gregorio T.A., Young H.A., Kasik J.W., Ozato K. and Papas T.S. (1989). Expression of *ets* genes in mouse thymocyte subsets and T cells. J. Immunol. 142, 672-678.

Bhat N.K., Thompson C.B., Lindsten T., June C.H., Fujiwara S., Koizumi S., Fisher R.J. and Papas T.S. (1990). Reciprocal expression of human ETS1 and ETS2 genes during T-cell activation. Proc. Natl. Acad. Sci. USA 87, 3723-3727.

Boise L.H., Gonzalez-Garcia M., Postema C.E., Ding L., Lindsten T., Turka L.A., Mao X., Nunez G. and Thompson C.B. (1993). *bcl-x*, a *bcl-2*-related gene that functions as a dominant regulator of apoptotic cell death. Cell 74, 597-608.

Bories J.C., Willerford D.M., Grevin D., Davidson L., Camus A., Martin P., Stehelin D. and Alt F.W. (1995). Increased T-cell apoptosis and terminal B-cell differentiation induced by inactivation of the ets-1 proto-oncogene. Nature 377, 635-638.

Boulukos K.E., Pognonec P., Begue A., Gesquière J.C., Stéhelin D. and Ghysdael J. (1988). Identification in chickens of an evolutionarily conserved cellular *ets*-2 gene (c-*ets*-2) encoding nuclear proteins related to the products of the c-*ets* proto-oncogene. EMBO J. 7, 697-705.

Boulukos K.E., Pognonec P., Rabault B., Begue A. and Ghysdael J. (1989). Definition of an Ets1 protein domain required for nuclear localization in cells and DNA-binding activity in vitro. Mol. Cell. Biol. 9, 5718-5721.

Broome H.E., Dargan C.M., Krajewski S. and Reed J.C. (1995). Expression of Bcl-2, Bcl-x, and Bax after T cell activation and IL-2 withdrawal. J. Immunol. 155, 2311-2317.

Chatterjee D., Han Z., Mendoza J., Goodglick L., Hendrickson E.A., Pantazis P. and Wyche J.H. (1997). Monocytic differentiation of HL-60 promyelocytic leukemia cells correlates with the induction of Bcl-x_L. Cell Growth Differ. 8, 1083-1089.

Chen C., Edelstein L.C. and Gelinas C. (2000). The Rel/NF-kappaB family directly activates expression of the apoptosis inhibitor Bcl-x(L). Mol. Cell. Biol. 20, 2687-2695.

Chen F., Demers L.M., Vallyathan V., Lu Y., Castranova V. and Shi X. (1999). Involvement of 5'-flanking kappaB-like sites within *bcl-x* gene in silica-induced *bcl-x* expression. J. Biol. Chem. 274, 35591-35595.

Dixon E.P., Stephenson D.T., Clemens J.A. and Little S.P. (1997). Bcl-Xshort is elevated following severe global ischemia in rat brains. Brain Res. 776, 222-229.

Dumon S., Constantino Rosa Santos S., Debierre-Grockiego F., Gouilleux-Gruart V., Cocault L., Boucheron C., Mollat P. and Gouilleux F. (1999). IL-3 dependent regulation of bcl-x_L gene expression by STAT5 in a bone marrow derived cell line. Oncogene 18, 4191-4199.

Fang W., Rivard J.J., Mueller D.L. and Behrens T.W. (1994). Cloning and molecular characterization of mouse *bcl-x* in B and T lymphocytes. J. Immunol. 153, 4388-4398.

Fujio Y., Kunisada K., Hirota H., Yamauchi-Takihara K. and Kishimoto T. (1997). Signals through gp130 upregulate *bcl-x* gene expression via STAT1-binding cis-element in cardiac myocytes. J. Clin. Invest. 99, 2898-2905.

Ghysdael J. and Boureux A. (1997). The ETS family of transcriptional regulators. In: Oncogenes as transcriptional regulators: Retroviral oncogenes. Yaniv M. and Ghysdael J. (eds) Birkhauser. Basel. pp 29-88.

Gibson L., Holmgreen S.P., Huang D.C.S., Bernard O., Copeland N.G., Jenkins N.A., Sutherland G.R., Baker E., Adams J.M. and Cory S. (1996). *bcl-w*, a novel member of the *bcl-2* family, promotes cell survival. Oncogene 13, 665-675.

Gonzalez-Garcia M., Perez-Ballestero R., Ding L., Duan L., Boise L.H., Thompson C.B. and Nunez G. (1994). bcl-X_L is the major *bcl-x* mRNA form expressed during mouse development and its product localizes in mitochondria. Development 120, 3033-3042.

Grillot D.A.M., Merino R. and Nunez G. (1995). Bcl-x_L displays restricted distribution during T cell development and inhibits multiple forms of apoptosis but not clonal deletion in transgenic mice. J. Exp. Med. 182, 1973-1983.

Grillot D.A., Merino R., Pena J.C., Fanslow W.C., Finkelman F.D., Thompson C.B. and Nunez G. (1996). *bcl-x* exhibits regulated expression during B cell development and activation and modulates lymphocyte survival in transgenic mice. J. Exp. Med. 183, 381-391.

Grillot D.A., Gonzalez-Garcia M., Ekhterae D., Duan L., Inohara N., Ohta S., Seldin M.F. and Nunez G. (1997). Genomic organization, promoter region analysis, and chromosome localization of the mouse *bcl-x* gene. J. Immunol. 158, 4750-4757.

Grossmann M., Metcalf D., Merryfull J., Beg A., Baltimore D. and Gerondakis S. (1999). The combined absence of the transcription factors Rel and RelA leads to multiple hemopoietic cell defects. Proc. Natl. Acad. Sci. USA 96, 11848-11853.

Hatada E.N., Krappmann D. and Scheidereit C. (2000). NF-kappaB and the innate immune response. Curr. Opin. Immunol. 12, 52-58.

Hettmann T., DiDonato J., Karin M. and Leiden J.M. (1999). An essential role for nuclear factor kappaB in promoting double positive thymocyte apoptosis. J. Exp. Med. 189, 145-158.

Iwanaga Y., Tsukahara T., Ohashi T., Tanaka Y., Arai M., Nakamura M., Ohtani K., Koya Y., Kannagi M., Yamamoto N. and Fujii M. (1999). Human T-cell leukemia virus type 1 tax protein abrogates interleukin-2 dependence in a mouse T-cell line. J. Virol. 73, 1271-1277.

Jacobs-Helber S.M., Wickrema A., Birrer M.J. and Sawyer S.T. (1998). AP1 regulation of proliferation and initiation of apoptosis in erythropoietin-dependent erythroid cells. Mol. Cell. Biol. 18, 3699-3707.

Jayaraman G., Srinivas R., Duggan C., Ferreira E., Swaminathan S., Somasundaram K., Williams J., Hauser C., Kurkinen M., Dhar R., Weitzman S., Buttice G. and Thimmapaya B. (1999). p300/cAMP-responsive element-binding protein interactions with Ets-1 and Ets-2 in the transcriptional activation of the human stromelysin promoter. J. Biol. Chem. 274, 17342-17352.

Jin D.I., Jameson S.B., Reddy M.A., Schenkman D. and Ostrowski M.C. (1995). Alterations in differentiation and behavior of monocytic phagocytes in transgenic mice that express dominant suppressors of ras signaling. Mol. Cell. Biol. 15, 693-703.

Kozopas K.M., Yaug T., Buchan H.L., Zhou P. and Craig R.W. (1993). MCL1, a gene expressed in programmed myeloid cell differentiation, has sequence similarity to BCL2. Proc. Natl. Acad. Sci. USA 90, 3516-3520.

Lee H.H., Dadgostar H., Cheng Q., Shu J. and Cheng G. (1999). NF-kappaB-mediated up-regulation of *bcl-x* and Bfl-1/A1 is required for CD40 survival signaling in B lymphocytes. Proc. Natl. Acad. Sci. USA 96, 9136-9141.

Lin E.Y., Orlofsky A., Wang H.G., Reed J.C. and Prystowsky M.B. (1996). A1, a Bcl-2 family member, prolongs cell survival and permits myeloid differentiation. Blood 87, 983-992.

Lotem J. and Sachs L. (1995). Regulation of bcl-2, bcl-X_L and *bax* in the

control of apoptosis by hematopoietic cytokines and dexamethasone. Cell Growth Differ. 6, 647-653.

Ma A., Pena J.C., Chang B., Margosian E., Davidson L., Alt F.W. and Thompson C.B. (1995). *bcl-x* regulates the survival of double positive thymocytes. Proc. Natl. Acad. Sci. USA 92, 4763-4767.

MacKichan M. and Israel A. (1997). Rel proteins and their inhibitors: a balancing act. In: Oncogenes as transcriptional regulators: Retroviral oncogenes. Yaniv M. and Ghysdael J. (eds.) Birkhauser. Basel. pp 165-221.

McKercher S., Torbett B., Anderson K., Henkel G., Vestal D., Baribault H., Klemsz M., Feeney A., Wu G., Paige C. and Maki R. (1996). Targetted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. EMBO J. 15, 5647-5658.

Motoyama N., Wang F., Roth K.A., Sawa H., Nakayama K., Negishi I., Senju S., Zhang Q., Fujii S. and Loh D.Y. (1995). Massive cell death of immature hematopoietic cells and neurons in Bcl-x-deficient mice. Science 267, 1506-1510.

Muthusamy N., Barton K. and Leiden J.M. (1995). Defective activation and survival of T cells lacking the ets-1 transcription factor. Nature 377, 639-642.

Nosaka T., Kawashima T., Misawa K., Ikuta K., Mui A.L. and Kitamura T. (1999). STAT5 as a molecular regulator of proliferation, differentiation and apoptosis in hematopoietic cells. EMBO J. 18, 4754-4765.

Packham G., White E.L., Eischen C.M., Yang H., Parganas E., Ihle J.N., Grillot D.A.M., Zambetti G.P., Nunez G. and Cleveland J.L. (1998). Selective regulation of Bcl-x_L by a Jak kinase-dependent pathway is bypassed in murine hematopoietic malignancies. Genes Dev. 12, 2475-2487.

Pfitzner E., Jahne R., Wissler M., Stoecklin E. and Groner B. (1998). p300/CREB-binding protein enhances the prolactin-mediated transcriptional induction through direct interaction with the transactivation domain of Stat5, but does not participate in the Stat5-mediated suppression of the glucocorticoid response. Mol. Endocrinol. 12, 1582-1593.

Pognonec P., Boulukos K.E., Gegonne A., Bailly M., Stéhelin D. and Ghysdael J. (1987). Identification and differential expression of the proteins encoded by the c-*ets*-1 and c-*ets*-2 proto-oncogenes. Arch. Int. Physiol. Biochem. 96, B51.

Pognonec P., Boulukos K.E., Gesquière J.C., Stéhelin D. and Ghysdael J. (1988). Mitogenic stimulation of thymocytes results in the calcium-dependent phosphorylation of c-Ets-1 proteins. EMBO J. 7, 977-983.

Quandt K., Frech K., Karas H., Wingender E. and Werner T. (1995). MatInd and MatInspector - New fast and versatile tools for detection of consensus matches in nucleotide sequence data. Nucl. Acids Res. 23, 4878-4884.

Scott E.W., Simon M.C., Anastasi J. and Singh H. (1994). Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. Science 265, 1573-1577.

Sevilla L., Aperlo C., Dulic V., Chambard J.C., Boutonnet C., Pasquier O., Pognonec P. and Boulukos K.E. (1999). The Ets2 transcription factor inhibits apoptosis induced by CSF-1 deprivation of macrophages through a Bcl-x_L-dependent mechanism. Mol. Cell. Biol. 19, 2624-2634.

Sevilla L., Zaldumbide A., Carlotti F., Abdel Dayem M., Pognonec P. and Boulukos K.E. (2001). Bcl-x_L expression correlates with primary macrophage differentiation, activation of functional competence and survival, and results from synergistic transcriptional activation by Ets2 and PU.1. J. Biol. Chem. (in press).

Shiraiwa N., Inohara N., Okada S., Yuzaki M., Shoji S.-I. and Ohta S. (1996). An additional form of Rat Bcl-x, Bcl-xß, generated by an unspliced RNA, promotes apoptosis in promyeloid cells. J. Biol. Chem. 271, 13258-13265.

Silva M., Benito A., Sanz C., Prosper F., Ekhterae D., Nunez G. and Fernandez-Luna J.L. (1999). Erythropoietin can induce the expression of bcl-x(L) through Stat5 in erythropoietin-dependent progenitor cell lines. J. Biol. Chem. 274, 22165-22169.

Socolovsky M., Fallon A.E., Wang S., Brugnara C. and Lodish H.F. (1999). Fetal anemia and apoptosis of red cell progenitors in Stat5a-/-5b-/- mice: a direct role for Stat5 in Bcl-X(L) induction. Cell 98, 181-191.

Stein B., Baldwin A.S. Jr., Ballard D.W., Greene W.C., Angel P. and Herrlich P. (1993). Cross-coupling of the NF-kappa B p65 and Fos/Jun transcription factors produces potentiated biological function. EMBO J. 12, 3879-3891.

Thomas R.S., Tymms M.J., McKinlay L.H., Shannon M.F., Seth A. and Kola I. (1997). ETS1, NFkappaB and AP1 synergistically transactivate the human GM-CSF promoter. Oncogene 14, 2845-2855.

Tran Quang C., Wessely O., Pironin M., Beug H. and Ghysdael J. (1997). Cooperation of Spi-1/PU-1 with an activated erythropoietin receptor inhibits apoptosis and Epo-dependent differentiation in primary erthyroblasts and induces their kit ligand-dependent proliferation. EMBO J. 16, 5639-5653.

Tsukahara T., Kannagi M., Ohashi T., Kato H., Arai M., Nunez G., Iwanaga Y., Yamamoto N., Ohtani K., Nakamura M. and Fujii M. (1999). Induction of Bcl-x(L) expression by human T-cell leukemia virus type 1 Tax through NF-kappaB in apoptosis-resistant T-cell transfectants with Tax. J. Virol. 73, 7981-7987.

Wasylyk B., Hahn S.L. and Giovane A. (1993). The Ets family of transcription factors. Eur. J. Biochem. 211, 7-18.

Yamamoto H., Kihara-Negishi F., Yamada T., Hashimoto Y. and Oikawa T. (1999). Physical and functional interactions between the transcription factor PU.1 and the coactivator CBP. Oncogene 18, 1495-1501.

Accepted November 23, 2000