CA Repeats in the 3′-Untranslated Region of bcl-2 mRNA Mediate Constitutive Decay of bcl-2 mRNA*

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An AU-rich element (ARE) in the 3′-untranslated region (UTR) of bcl-2 mRNA has previously been shown to be responsible for destabilizing bcl-2 mRNA during apoptosis through increasing AUF1 binding. In the present study, we investigated the effect of the region upstream of the ARE on bcl-2 mRNA stability using serial deletion constructs of the 3′-UTR of bcl-2. Deletion of 30 nucleotides mostly consisting of the CA repeats, located upstream of the ARE, resulted in the stabilization of bcl-2 mRNA abundance, in the absence or presence of the ARE. The specificity of the CA repeats in terms of destabilizing bcl-2 mRNA was proven by the substituting the CA repeats with other alternative repeats of purine/pyrimidine, but this had no effect on the stability of bcl-2 mRNA. CA repeats alone, however, failed to confer instability to bcl-2 or gfp reporter mRNAs, indicating a requirement for additional sequences in the upstream region of the 3′-UTR. Serial deletion and replacement of a part of the region upstream of the CA repeats revealed that the entire 131-nucleotide upstream region is an essential prerequisite for the CA repeat-dependent destabilization of bcl-2 mRNA. Unlike the ARE, CA repeat-mediated degradation of bcl-2 mRNA was not accelerated upon apoptotic stimulus. Moreover, the upstream sequences and CA repeats are conserved among mammals. Collectively, CA repeats contribute to the constitutive decay of bcl-2 mRNA in the steady states, thereby maintaining appropriate bcl-2 levels in mammalian cells.

Apoptosis is a tightly controlled cellular suicide program that is critical for the successful development of multicellular organisms, the maintenance of normal tissue homeostasis, and removal of damaged cells (1). The protooncogene bcl-2, originally isolated from the chromosomal breakpoint of a t(14, 18)-bearing B cell lymphoma, serves as an important repressor of apoptosis in a variety of cell types (2, 3). In line with its significant role in altering susceptibility to apoptosis, investigations of the mechanisms by which bcl-2 expression is modulated may prove crucial for identifying therapeutic strategies for cancer and some neurodegenerative diseases and for defining the role of bcl-2 in the development of multiple tissues (4).

Recent studies have indicated that bcl-2 is regulated at both the transcriptional and posttranscriptional levels. A number of negative transcriptional regulatory sites have been described in the bcl-2 promoter region (5, 6), and several transcription factors, including cAMP response element binding protein, A-Myb and WT1, are known to be involved in the positive regulation of bcl-2 transcription (7–9). In addition to the promoter region, some sequences within the coding region, such as estrogen response elements, have also been demonstrated to mediate the transcriptional modulation of bcl-2, as was shown in breast cancer cell lines (10). The posttranscriptional modification of bcl-2 includes the phosphorylation of Bcl-2 at the putative mitogen-activated protein kinase sites, which confers resistance against ubiquitination- and proteasome-dependent degradation (11, 12), and caspase-dependent cleavage, which results in the loss of anti-apoptotic activity (13, 14). Another mechanism of posttranscriptional regulation of bcl-2 expression is based on mRNA stability. Recent reports have described that a conserved AU-rich element (ARE) (15) is present in the 3′-untranslated region (UTR) of bcl-2 mRNA (15) and that interaction of ARE with a number of ARE-binding proteins, including AUF1, is associated with bcl-2 mRNA decay during apoptosis (16, 17). In addition, transfection of a synthetic ribozyme targeting bcl-2 ARE was shown to successfully down-regulate bcl-2 mRNA expression accompanied by an increase in cell death in lymphoma cells (18), thus supporting the central role of ARE in the regulation of bcl-2 gene expression.

A recent study using a cell-free in vitro degradation assay revealed that a 396-nt segment of the bcl-2 3′-UTR including the ARE was degraded faster than the ARE motif itself and that the 3′-UTR lacking the ARE was still degraded faster than control RNA (19). These findings suggest that, in addition to the ARE, other regulatory sites might exist in the 3′-UTR, upstream or downstream of the ARE. Here, we investigated the effect of the region upstream of the ARE on bcl-2 mRNA stability and identified a new putative bcl-2 mRNA destabilization determinant that contains CA repeats, mediating the constitutive decay of bcl-2 mRNA.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—The African green monkey kidney cells (COS7) were obtained from the American Type Culture Collection

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‡ The abbreviations used are: ARE, AU-rich element; 3′-UTR, 3′-untranslated region; CAR, CA-repeated region; Act D, actinomycin D; GFP, green fluorescence protein; DIG, digoxigenin; n.t. nucleotide(s); GAFDH, glyceraldehyde-3-phosphate dehydrogenase; eNOS, endothelial nitric-oxide synthase.
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and grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum (HyClone) and antibiotic solutions (100 units/ml penicillin and 100 μg/ml streptomycin, Invitrogen) under a 5% CO2 humidified atmosphere at 37 °C. COS7 cells (1 × 10⁶) were transfected in 6-well plates with 1 μg of plasmids using FuGENE 6 reagent (Roche Applied Science) according to the manufacturer's instructions. COS7 cells were harvested at 48 h after transfection for Northern and Western blotting. To evaluate mRNA degradation rates, further transcription was inhibited by adding actinomycin D (Act D) at 10 μg/ml (Sigma) 24 h after transfection, and then further incubating cells, before total RNA was extracted. To determine the degradation rate of bcl-2 mRNA under apoptotic condition, 1 ml H2O2 was added to COS7 cells 1 h prior to treating Act D.

Preparation of plasmids—A 1114 nt fragment for the BU4 plasmid including the coding region (1459–2178) and 394 nt of the 3'-UTR was first obtained by PCR amplification using cDNA from HL60 cells as a template and corresponding primers flanked by an EcoRI restriction site (see Fig. 1A; all nucleotide positions were based on the sequence of accession number M13994 in GenBankTM). The PCR products were purified from a 1% agarose gel and inserted into the TA cloning site of pGEM-T easy vector (Promega); sequences were confirmed by automated DNA sequencing. The EcoRI fragment was then subcloned in the EcoRI site of pCR3.1, named BU4. Other bcl-2 cDNA constructs containing various lengths of the 3'-UTR were also produced by PCR using BU4 as a template for B, BU1, BU2, and BU3 and using BU5 for BU4-1, BU4-2, BU4-3, and BU4-4 with corresponding primers. PCR products were attached by other alternative repeats of purine and pyrimidine (BU2-2AT, BU2-2CG, BU2-2GT and BU2-2CA), reverse primers were designed to include five respective repeats at the last part, and BU2-2 was used as a template.

To prepare a chimeric dRNA, which contains a coding region of bcl-2 and the 3'-UTR of unrelated gene followed by CA repeats (BU3n), two separate PCR amplifications were performed as follows. The first part confining the 3'-bcl2 coding region was synthesized with 5'-GAATTCAT-GGCCACGCTGGGAAGAGGGG-3' (forward, primer1) and 5'-GCT-GACTGCTACTGTGCTAGATAGGCAAC-3' (reverse) flanked at the 5'-end by EcoRI and Nhel restriction sites, respectively. The second part containing the 3'-UTR on the transcription of bcl-2 were also produced by PCR using BU5 for BU5-1, BU5-2, BU5-3, BU5-4 with corresponding primers. PCR products were attached by other alternative repeats of purine and pyrimidine (BU2-2TGTGACGTGACG-3, BU3-2TGTGACGTGACG-3 and BU4-2TGTGACGTGACG-3), reverse primers were designed to include five respective repeats at the last part, and BU2-2 was used as a template.

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To determine the rate of bcl-2 mRNA decay, total RNA was isolated from cells at multiple time points after treatment with Act D and then subjected to Northern blot analysis as described above. The membrane was stripped and rehybridized with GAPDH probe. The bcl-2 mRNA signal was quantified at each time point by densitometry using Scion image software (Scion Corp.), normalized to GAPDH, and plotted as a percentage of the initial value against time.

Immunoassay for Bcl-2 and Actin Proteins—Cells were lysed in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM sodium chloride, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate), and equal amounts of protein were separated by 12.5% SDS-PAGE followed by electrotransfer onto a polyvinylidene difluoride membrane (Millipore). The membranes were incubated with a monoclonal antibody to human Bcl-2 (Santa Cruz Biotechnology), GFP (Clontech), or β-actin (Sigma) and immunolabeling was done with horseradish peroxidase-conjugated secondary antibodies (Promega) using the enhanced detection system (Amersham Biosciences).

In Vitro Transcription—To examine the expression of the CA repeats in the 3’-UTR on the transcription of bcl-2, the transcripts of BU2 or BU3 constructs were synthesized in vitro using T7 RNA polymerase (Roche Applied Science) in transcription reaction buffer containing 20 units of RNase inhibitor and a 0.05 mM concentration each of ATP, CTP, GTP, and UTP. Twenty microliters of transcription reaction mixture were incubated at 37 °C according to the manufacturer's instruction. After 30 min, the transcripts were reverse-transcribed with Murine Moloney Murine leukemia virus (MMLV) reverse transcriptase (Promega, 1 unit/reaction), the purity as well as the amount of RNA transcripts were determined by using 1% formaldehyde-agarose gel followed by ethidium bromide staining.

RESULTS

The CA Repeats in the 3’-UTR Contribute to the Destabilization of bcl-2 mRNA—To investigate the contribution of the sequence of the 3'-UTR upstream of the ARE of bcl-2 to the stability of bcl-2 mRNA, we prepared a series of 3’-UTR deletion mutants of bcl-2 between its stop codon and the ARE by PCR, as shown in Fig. 1A. Each cDNA fragment-containing coding region and the indicated portion of the 3'-UTR was inserted into the pCR3.1 vector. The resulting constructs were transiently transfected into COS7 cells, and the level of bcl-2 expression from each construct was analyzed by Northern and Western blotting. Fig. 1B shows that no significant differences of bcl-2 expression were found in cells transfected with B, BU1, or BU2. However, the bcl-2 mRNA level was lower in cells transfected with BU3 cDNA than in cells transfected with BU1, BU2 or BU3, which indicates that the presence of the CA-rich CA repeats in the 3’-UTR of bcl-2 mRNA is necessary for the ARE to respond to RNase inhibitor and a 0.05 mM concentration each of ATP, CTP, GTP, and UTP. Twenty microliters of transcription reaction mixture were incubated at 37 °C according to the manufacturer's instruction. After 30 min, the transcripts were reverse-transcribed with Murine Moloney Murine leukemia virus (MMLV) reverse transcriptase (Promega, 1 unit/reaction), the purity as well as the amount of RNA transcripts were determined by using 1% formaldehyde-agarose gel followed by ethidium bromide staining.

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vector, which has a cytomegalovirus promoter, the difference in the bcl-2 mRNA level between BU2 and BU3 constructs was likely to be due to the differences in mRNA stability rather than differences in the transcription rate. Supporting our presumption, in vitro transcription experiments using T7 polymerase and BU2 or BU3 as templates revealed no significant difference in transcript level from each template (Fig. 1C). We then compared the mRNA degradation rates of the transcripts from these two constructs by Act D chase experiments. Fig. 1D shows that the mRNA level of BU3 was reduced to 29% of the control at 5 h after adding Act D, while the mRNA level of BU2 was maintained at 56% of the control level, indicating that the BU3 mRNA was degraded faster than the BU2 mRNA. Therefore, these results suggest that the part of the 3′-UTR region present in BU3 but deleted in BU2 has the potential to affect bcl-2 mRNA stability rather than the transcription processes.

To identify in detail the sequences with the mRNA destabilizing potential, the last 50 nt of the 3′-UTR sequences of the BU3 cDNA were serially deleted in 10-nt increments, and the expression levels of each construct were then compared by Northern blotting. As shown in Fig. 2B, transfection of the BU2-1 or BU2-2 construct, which, respectively, has 10 or 20 bases more than the BU2 construct, did not cause any decrease in the bcl-2 mRNA level compared with BU2 cDNA. However, the mRNA level gradually decreased in the cells transfected with BU2-3, BU2-4, or BU3, which had serial additions of 10 nt, up to 30 nt. Interestingly, the sequences of these 30 nt (2311–2340), which dramatically reduced the bcl-2 mRNA level, are mostly composed of CA repeats (+2 GA), indicating
that bcl-2 mRNA stability decreased in proportion to the length of the CA repeats. We designated this region as CAR. It is notable that the BU2-3 construct, which has only five CA repeats, significantly reduced the bcl-2 mRNA level. When the five repeats of CA in the BU2-3 cDNA were replaced with other purine-pyrimidine repeated sequences, BU2, BU2-1, BU2-2, BU2-3, BU2-4, and BU3 (B) and from BU2-2, BU2-2_A, BU2-2_C, BU2-2_G, and BU2-2_A (C). Transfection and detection were performed as described in the legend to Fig. 1.

Sequences Upstream of the 3'-UTR Are Required for CA Repeat-dependent Destabilization of bcl-2 mRNA—To establish whether the presence of CA repeats alone is sufficient to regulate bcl-2 mRNA stability, we made two chimeric constructs, termed BU3bis and BCAR, respectively, as indicated in Fig. 3A. Transfection with the BCAR construct, in which 131 nt of the bcl-2 3'-UTR upstream of CA repeats were deleted, resulted in a marked stabilization of bcl-2 mRNA despite the presence of the CA repeats, indicating an additional requirement of the proximal sequences of 3'-UTR for the CA repeat-dependent destabilization of bcl-2 mRNA. Replacement of the proximal 131 nt of the bcl-2 3'-UTR with the unrelated sequences of 131 nt of the bis 3'-UTR also failed to destabilize bcl-2 mRNA as efficiently as the 3'-UTR of bcl-2, even though a slight destabilization was observed. Therefore, it is not the simple distance between the stop codon and the CA repeats but the specific sequences in the 131 nt of the 3'-UTR that seem to confer the destabilizing potential of the CA repeats.

The destabilizing activity of the proximal region of the 3'-UTR of bcl-2 was confirmed using a GFP reporter gene. The 3'-end of the open reading frame of the GFP cDNA was linked to the 131 nt of the 3'-UTR of bcl-2 and subsequently to 30 nt of CA repeats, or directly to the CA repeats only, as described under "Experimental Procedures." Compared with the mRNA level expressed from the GFP coding region only, the insertion of 131 nt of the 3'-UTR together with CA repeats into the GFP coding region resulted in a comparable decrease in the GFP mRNA level, whereas insertion of the CA repeats only caused no decrease (Fig. 3B). The incorporation of 131 nt of the 3'-UTR of the bis gene, instead of the 3'-UTR of bcl-2, had no effect on gfp mRNA stability as in bcl-2 mRNA, verifying the potential destabilizing activity of the 3'-UTR of bcl-2, which demands both the proximal 3'-UTR of bcl-2 and subsequent CA repeats.

To determine the essential region affecting the CA repeat-dependent bcl-2 mRNA destabilization, we attached 30 nt of CA repeats to 42 or 82 nt of the 3'-UTR of bcl-2. The bcl-2 mRNA expressed from these two chimeric constructs showed significantly high levels, which corresponded to the mRNA levels from the construct in which all 131 nt were deleted (Fig. 4A). These results suggest that the last part of the 131 nt, lacking in these two constructs but present in the BU3 wild type, might be important for the CA repeat-dependent destabilization of bcl-2 mRNA.

Subsequently, the last part of the proximal 131 nt of the 3'-UTR, as well as the first and middle part, were substituted with unrelated sequences. As shown in Fig. 4B, the replacement of each part increased bcl-2 mRNA stability to a level similar to that of construct B, which contains only the coding region (Fig. 4B). These results, taken together, indicated that the overall mRNA structure provided by the linear sequences of the complete 131 nt of the 3'-UTR might be a prerequisite for the destabilizing ability of the subsequent CA repeats.

CA Repeat-dependent bcl-2 mRNA Destabilization Is Not Affected by Apoptosis—It has been reported previously that ARE-mediated decay in bcl-2 mRNA is enhanced during apoptosis and that this is accompanied by increased binding of several AUFs (15–17). To determine whether CA repeat-mediated bcl-2 mRNA degradation also participates in the down-modulation of Bcl-2 during apoptosis, we investigated whether the degradation rate of bcl-2 mRNAs with the 3'-UTR including the CA repeats but lacking ARE is affected by apoptotic stimulation. Act D chase experiments up to 5 h following transient transfection for 24 h revealed that the degradation of bcl-2 mRNA with both CA repeats and ARE (BU4) was significantly accelerated by \( \text{H}_2\text{O}_2 \) treatment (Fig. 5, B and central panel of
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mRNA decay is also modulated in part by the CA repeats in the 3'-UTR, which are located about 131 nt from the stop codon and upstream of the previously characterized ARE. The destabilizing potential of the CA repeats was confirmed by deleting the CA repeats from the 3'-UTR of bcl-2, which resulted in a marked increase in the level of bcl-2 mRNA despite the presence of the ARE sequence (Fig. 5). These results suggest that the CA repeat is a novel determinant of bcl-2 mRNA decay, which is in line with the previous finding that an ARE-deficient 3'-UTR of bcl-2 was still degraded faster than control 3'-UTR in vitro, suggesting the presence of an additional bcl-2 mRNA decay-regulatory element in the 3'-UTR (19). Therefore, the destabilizing activity of the 3'-UTR of bcl-2 including the ARE demonstrated in earlier studies, which primarily focused on the ARE as a representative bcl-2 mRNA destabilizing determinant, seems to be attributable not entirely to the ARE but substantially to CA repeats as well.

CA repeats are the most common dinucleotide polymorphism found in the human genome and thus are routinely used as genetic markers of allelic variants in various genes (22–24). Recently, a number of reports have described a correlation between the reduced expression levels of several genes such as IFN-γ and HSD11B2 and either the presence of repeats or an increase in the length of the repeats in the intron, suggesting a regulatory function of CA repeats on the processing of pre-mRNAs (25–27). Furthermore, CA repeats in intron 13 of the human endothelial nitric-oxide synthase (eNOS) gene seem to specify the cleavage site of the pre-mRNA of eNOS for splicing or degradation, depending on the presence of heterogeneous nuclear ribonucleoprotein L (hnRNPL) (28, 29). However, little is known about the functional importance of CA repeats in the exonic context. Here we describe for the first time that CA repeats in the 3'-UTR can target bcl-2 mRNA for selective degradation, extending the role of CA repeats as an important element for modulating the stability of mRNA as well as the stability of pre-mRNA.

The mechanism by which CA repeats in an exon exert a negative effect on bcl-2 mRNA abundance has not been clarified. However, exonic CA repeats for mRNA destabilization appear to involve a different pathway from that of intronic CA repeats for specifying the cleavage sites of the pre-RNA of eNOS, given that the CA repeats did not function as a splicing enhancer when moved in an exonic context (28). Furthermore, CA repeats were shown to be sufficient for the cleavage of eNOS pre-mRNA (29), whereas CA repeats were found to be essential but not sufficient to destabilize bcl-2 mRNA, as shown by our results indicating that the incorporation of the CA repeats directly into the 3'-end of coding sequences of bcl-2 or GFP mRNA did not affect mRNA stability (Fig. 3, A and B). On the other hand, levels of GFP reporter gene mRNA decreased after introduction of the proximal 131 nt and subsequent CA repeats of the 3'-UTR of bcl-2, whereas the incorporation of the same length of the 3'-UTR of the bcl-2 gene with the CA repeats had no effect on GFP or bcl-2 mRNA stability. Finally, our deletion and replacement experiments indicate that the entire 131 nt region preceding the CA repeats is apparently a requisite for the destabilizing activity of the CA repeats (Fig. 4, A and B).

In addition to the requirement of the upstream sequences of the 3'-UTR, CA repeat-mediated decay of bcl-2 mRNA was found to exhibit features that differed from ARE-mediated processes in several respects. The ARE in the 3'-UTR of bcl-2 has been known to mediate bcl-2 mRNA decay during apoptosis, either by increasing binding of destabilizing factors such as AUF1 (16, 17) or by reducing interaction with stabilizing factors such as nucleolin (30, 31). Our results show that the decay
rate of the \textit{bcl-2} mRNA from the construct including the CA repeats but not the ARE was not significantly changed upon apoptosis induction, whereas \textit{bcl-2} mRNA from the construct including the ARE and CA repeats was degraded faster under the same conditions (Fig. 5). Moreover, the transcript from the \textit{bcl-2} DNA including the ARE but devoid of the CA repeats showed increased stability in nonapoptotic conditions but accelerated degradation in apoptotic conditions. Therefore, CA repeat-mediated decay of \textit{bcl-2} appears to contribute to maintaining appropriate Bcl-2 levels in the steady states, while the ARE-dependent destabilizing pathway may react to apoptotic stimuli to permit a rapid down-regulation of the Bcl-2 level.

\textbf{FIG. 5.} CA repeat-dependent decay of \textit{bcl-2} mRNA is not enhanced by apoptosis. COS7 cells were transiently transfected with three \textit{bcl-2} constructs including CAR (BU3), ARE only (BU4\textsubscript{CAR}), or both (BU4) in the 3′-UTR as shown in A, and \textit{bcl-2} mRNA stability was analyzed in the presence of Act D. To induce apoptosis, H\textsubscript{2}O\textsubscript{2} (1 mM) was added to cells 1 h prior to adding Act D (10 μg/ml). Total RNA was extracted at 0, 1, 3, or 5 h after Act D treatment and analyzed by Northern blot for \textit{bcl-2} and subsequently for GAPDH as shown in B. C, \textit{bcl-2} mRNA signals derived from the three constructs (BU3, BU4, and BU4\textsubscript{CAR}) under normal and apoptotic condition were normalized with GAPDH mRNA signal from each lane in B after being quantified by densitometry using Scion image software (Scion Corp.). All data are represented as means ± S.D. from three independent experiments. \textit{bcl-2} mRNA signal without Act D was defined as 100% and Act untreated \textit{bcl-2} mRNA levels were calculated as percentages of decay at the indicated times.

\textbf{FIG. 6.} The nucleotide sequences and destabilization potential of an upstream portion of the 3′-UTR and CA repeats of the \textit{bcl-2} gene are conserved in mammals. A, the nucleotide sequences of the region upstream of the CA repeats in the 3′-UTR of \textit{bcl-2} gene were compared for human, rat, and mouse (GenBank\textsuperscript{TM} accession numbers on the left). Alignment was performed starting from the first nucleotide of the stop codon up to the last nucleotide of the CA repeats. The shared nucleotides among these three species are in bold and underlined. Multiple alignment comparison was performed using the ClustalW engine (www.justbio.com/aligner/). B, three constructs of mouse \textit{bcl-2}, prepared as shown in the left panel, were transfected into COS7 cells, and \textit{bcl-2} mRNA levels were analyzed (right panel).
thereby leading to cell death. However, more diverse apoptotic stimuli should be applied to confirm the relevance of these two cis-elements in Bcl-2 down-regulation in apoptosis. Interestingly, the presence of Bcl-2 protein was previously proposed as an essential requirement for the activation of ARE-dependent degradation programs (19). We also found that the destabilizing effect induced by the incorporation of the upstream sequences and CA repeats of the 3'-UTR of bcl-2 on GFP mRNA was less prominent than that of bcl-2 mRNA (Fig. 3), raising the possibility that CA repeat-mediated decay also entails the presence of Bcl-2 protein. However, the coexpression of Bcl-2 did not influence the level of GFP mRNA with or without the 3'-UTR of the bcl-2 gene in our study (data not shown). The amount of Bcl-2 protein was therefore not likely to be a critical prerequisite for the CA repeat-mediated decay of bcl-2 mRNA, but another cis-element in the coding region of bcl-2 might participate in the maintenance of the steady-state level of bcl-2 mRNA. Another difference is that the presence of CA repeats in the 3'-UTR of bcl-2 is confined to mammals, including humans, rats, and mice, whereas ARE is widely preserved, even being present in the 3'-UTR of nematode bcl-2 (15). Moreover, the sequences 5'-upstream of the CA repeats also reveal a high level of homology in mammalian bcl-2 (Fig. 6). Thus, CA repeat-mediated decay systems might have developed during evolutionary division into mammals to tune the bcl-2 level more finely. The presence of diverse modes of regulating the Bcl-2 level, as shown in our report and in previous reports (15–17), suggests that different mechanisms are involved in the modulation of the Bcl-2 level in response to different physiological and pathological conditions.

Most of the mechanisms that regulate mRNA stability involve specific interactions between structural determinants on mRNA, cis-acting elements and proteins that bind the determinants, trans-acting proteins, which modulate the susceptibility of mRNA to degradation (32). Cis-acting elements could be an actual target site for ribonuclease, or they might regulate ribonuclease attack elsewhere in the mRNA by binding with either stabilizing or destabilizing factors. It has been shown previously that CA repeats or CA-rich sequences have the potential to bind proteins such as heterogeneous nuclear ribonucleoprotein (hnRNP) in the intron of eNOS pre-mRNA or in the 3'-UTR of vascular endothelial growth factor (VEGF) mRNA (28, 39). Therefore, defining whether a protein binds to the upstream sequences or CA repeats in the 3'-UTR of bcl-2 and subsequently affects the bcl-2 mRNA stability will be a subject of further investigation to get a complete understanding of the process of destabilizing bcl-2 mRNA.

In conclusion, we describe a novel pathway of constitutive decay of bcl-2 mRNA that involves CA repeats and their upstream sequences in the 3'-UTR, extending the functional significance of CA repeats from an intronic to an exonic context. Further investigation on the molecular mechanisms by which the CA repeats exert their destabilizing activity, including RNA/protein interactions, may contribute to the development of new strategies for reducing Bcl-2 levels in pathological conditions.

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