

Database tool

BCL2DB: database of BCL-2 family members and BH3-only proteins

Valentine Rech de Laval¹, Gilbert Deléage¹, Abdel Aouacheria^{2,*} and Christophe Combet^{1,*}

¹Unité Bases Moléculaires et Structurales des Systèmes Infectieux, UMR 5086 CNRS - Université Claude Bernard Lyon 1, IBCP - 7, passage du Vercors, 69367 Lyon cedex 07, France and ²Molecular Biology of the Cell Laboratory, Ecole Normale Supérieure de Lyon, LBMC UMR 5239 CNRS - UCBL - HCL - ENS Lyon, 46 Allée d'Italie, 69364 Lyon Cedex 07, France

Corresponding author: Tel: +33 4 37 65 29 47; Fax: +33 4 72 72 26 01; Email: christophe.combet@ibcp.fr

Correspondence may also be addressed to Abdel Aouacheria. Tel: +33 4 26 23 59 43; Fax: +33 4 26 23 59 01; Email: abdel.aouacheria@ens-lyon.fr

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BCL2DB (<http://bcl2db.ibcp.fr>) is a database designed to integrate data on BCL-2 family members and BH3-only proteins. These proteins control the mitochondrial apoptotic pathway and probably many other cellular processes as well. This large protein group is formed by a family of pro-apoptotic and anti-apoptotic homologs that have phylogenetic relationships with BCL-2, and by a collection of evolutionarily and structurally unrelated proteins characterized by the presence of a region of local sequence similarity with BCL-2, termed the BH3 motif. BCL2DB is monthly built, thanks to an automated procedure relying on a set of homemade profile HMMs computed from seed reference sequences representative of the various BCL-2 homologs and BH3-only proteins. The BCL2DB entries integrate data from the Ensembl, Ensembl Genomes, European Nucleotide Archive and Protein Data Bank databases and are enriched with specific information like protein classification into orthology groups and distribution of BH motifs along the sequences. The Web interface allows for easy browsing of the site and fast access to data, as well as sequence analysis with generic and specific tools. BCL2DB provides a helpful and powerful tool to both 'BCL-2-ologists' and researchers working in the various fields of physiopathology.

Database URL: <http://bcl2db.ibcp.fr>

Introduction

Two distinct groups of BCL-2-related proteins control the mitochondrial apoptotic pathway and probably other cellular processes as well (1, 2). The first group is formed by a family of homologs related to BCL-2 by a common ancestry, and the second group comprises a heterogeneous collection of evolutionarily and structurally unrelated proteins characterized by the presence of a single short stretch of sequence similarity with BCL-2, termed the BH3 motif.

BCL-2 homologous proteins share a similar α -helical bundle fold (the 'BCL-2 domain'), have up to four different BH motifs (BH1-BH4) and can be either anti-apoptotic (e.g. BCL-2 and BCL-xL) or pro-apoptotic (e.g. Bax, Bak and Bid), whereas all of the BH3-only proteins are pro-apoptotic. Moreover, a variety of viral proteins have been found

to be structurally similar to BCL-2 with or without obvious sequence similarity (3).

Since the discovery of the *bcl-2* gene 30 years ago, intense research in various disciplines has exponentially increased the quantity of data available on the BCL-2 family and BH3-only proteins. Therefore, it is of considerable interest to use bioinformatic tools to (i) understand the various groups of proteins structurally or functionally linked to BCL-2 and their implication in diseases; (ii) bring all the available information together in a specialized database [for which we have previously developed a prototype (4)]. We recently proposed a novel classification scheme for BCL-2-related proteins, based on phylogenetic information and computational analysis of sequence data (5, 6). Here, we describe an enhanced version of the BCL-2 database, a

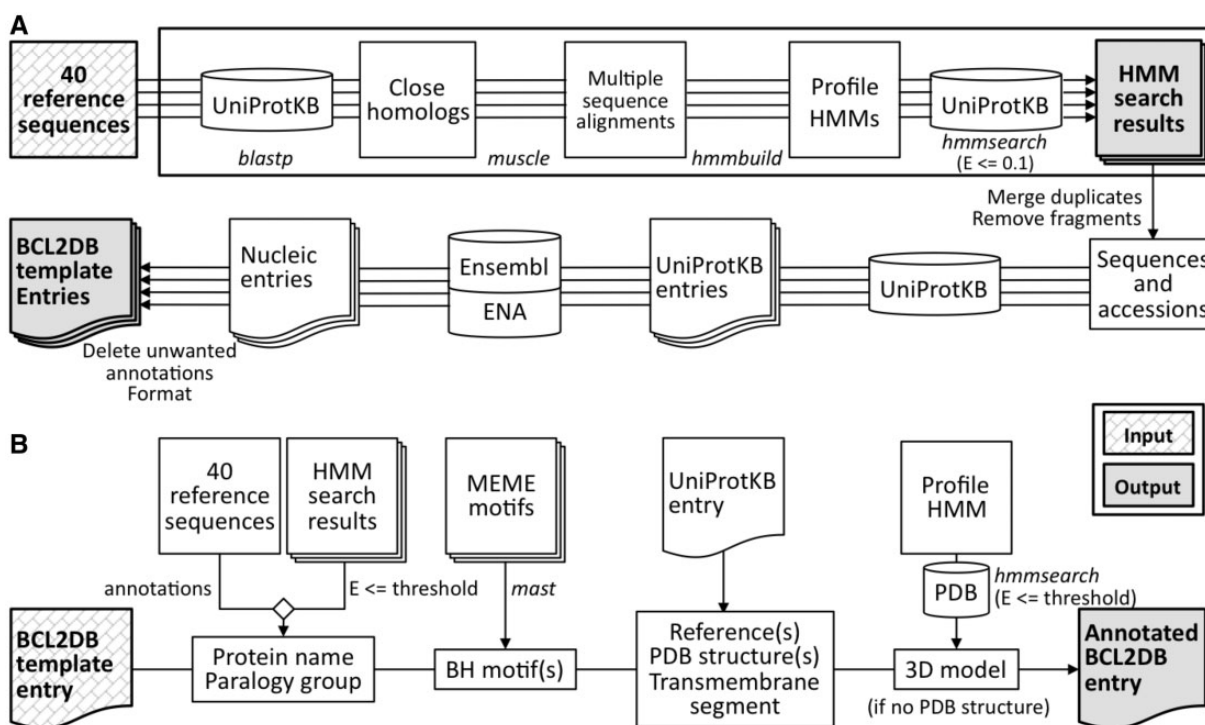


Figure 1. Description of the *FindBCL2* and *AnnotateBCL2* processes used to generate BCL2DB. External programs used by the processes are indicated in italics. **(A)** The upper part of the panel (boxed) describes the discovery mode of the *FindBCL2* program. The results are the profile HMMs and their associated classification E-value thresholds deduced after a HMM search against UniProtKB. The production mode used to generate the BCL2DB entry templates is described in the bottom part. After an *hmmsearch* on UniProtKB with the computed profile HMMs, the Ensembl or ENA entries are retrieved from cross-references or BLAST searches with nonfragment protein sequences and after removing duplicated sequences. Then, the entries are cleared of unwanted annotations and merged into a single one if they refer to the same Ensembl, Ensembl Genomes or ENA entry. **(B)** The *AnnotateBCL2* process enriches each BCL2DB entry template with annotations from reference sequences, sequence classification information (protein/gene name and orthology group/cluster), location of BH motifs and structural data retrieved from the PDB.

computer-annotated sequence database dedicated to BCL-2 homologous and BH3-only proteins, as well as the integrated Web interface that provides easy and efficient access to the data.

The BCL2DB database

BCL2DB is available since July 2013. The release 2 comprises 1039 entries, including 880 BCL-2 homologous proteins (655 encoded by metazoan genomes and 225 from viruses) and 159 BH3-only proteins. Based on our new classification scheme, we built an automated workflow to feed BCL2DB. The workflow relies on a set of specific profile HMMs (7) derived from 40 reference protein sequences representative of the various orthologous subgroups present within the BCL-2-like and BH3-only groups. This computational pipeline was able to identify both close and distant homologs of BCL-2 (including viral members) as well as the known repertoire of BH3-only proteins when searching the UniProt Knowledgebase (UniProtKB) (8). The identified sequences are then annotated to provide entries in

the European Nucleotide Archive (ENA) (9) EMBL-Bank format, which is loaded into a PostgreSQL relational database management system. Finally, sequence data sets are extracted and multiple sequence alignments are computed together with associated data. BCL2DB is updated on a monthly basis. All the programs of the computational pipeline have been written in Java, and SQL was used for database queries.

Identification of BCL-2 homologous sequences and BH3-only sequences

The *FindBCL2* program (Figure 1A) ensures sequence identification and provides two modes of execution: discovery and production. In the discovery mode, a profile HMM is computed (*hmmbuild* program of HMMER package 3.0) for each reference sequence (of individual BCL-2 homologs or BH3-only proteins) from a multiple alignment of their closest homologous sequences extracted after a BLAST search against UniProtKB with a score threshold tailored for each reference sequence. Each profile HMM is then used to search UniProtKB (*hmmsearch* program), and an E-value

Cellular

The repertoire of cellular BCL-2 family proteins contains several groups of paralogs. Below are listed the well-annotated groups data listed in the tables refers to human proteins unless otherwise noted.

BCL-2-like

Gene name	Protein name	Synonyms	Primary function	BCL2DB acc.	Reference
BCL-2	Bcl-2	-	inhibitor	P10415	Tsujimoto et al., 1984
BCL2L1	Bcl-xL	Bcl2L1	inhibitor	Q07817	Boise et al., 1993
BCL2L2	Bcl-w	Bcl2L2	inhibitor	Q92843	Gibson et al., 1996
MCL-1	Mcl-1	Bcl2L3	inhibitor	Q07820	Kozopou et al., 1993
BCL2L10	Bcl2L10	Bcl-B, Nrh, Nr-13, Diva, Boo	inhibitor	Q9HD38	Aouachria et al., 2001
BCL2A1	BH1	Bcl2a1, Bcl25	inhibitor	Q16548	

BAX-like

Gene name	Protein name	Synonyms	Primary function	BCL2DB acc.	Refer
BAX	Bax	Bcl24	promoter	Q07812	Oltvai et al.
BAK1	Bak1	Bcl27	promoter	Q16611	Chittenden/Inohara et al.
BOK	Bok	Bcl29, Mtd	promoter	Q9UMX3	
BCL-WAV	Bcl-WAV	-	uncharacterized	D2Y5Q2	Prudent et al.

BID-like

Gene name	Protein name	Synonyms	Primary function	BCL2DB acc.	Ref
BID	Bid	Bid	promoter	P55957	Wang et al.
BCL2L12	Bcl2L12	Bpr	promoter	Q9HB09	Scorilas
BCL2L13	Bcl2L13	Bcl-rambo, M81	promoter	Q9BXK5	Kataoka
BCL2L14	Bcl2L14	Bcl-G	promoter	Q9BZR8	Guo et al.
BCL2L15	Bcl2L15	Bfk	promoter	Q5TBC7	Coutas

Resources

- mat_peptide
- PRABI_prodfit
- mRNA
- GO
- PDB

PubMed

Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Science. 1984 Nov 30;226(4678):1097-9.

Abstract

From an acute B-cell leukemia cell line, a DNA probe was obtained that was specific for chromosome 18 and flanked the heavy chain joining region of the immunoglobulin heavy chain locus on chromosome 14. This probe detected rearrangement of the homologous DNA segment in the leukemic cells and in follicular lymphoma cells with the t(14;18) chromosome translocation but not in other neoplastic or normal B or T cells. The probe appears to identify bcl-2, a gene locus on chromosome 18 (band q21) that is unrelated to known oncogenes and may be important in the pathogenesis of B-cell neoplasms with this translocation.

Figure 2. Example of general information available for BCL2DB reference sequences. (A) Cellular BCL-2 homologous reference sequences general information ordered by main clades (BCL-2-like, BAX-like and BID-like) and organized as tables. For each reference sequence, two links are provided to view the BCL2DB entry and the PubMed entry of the article describing the protein discovery. (B) Partial view of the feature table corresponding to the BCL2DB entry P10415. Links are provided to retrieve nucleotide (e.g. mRNA or mat_peptide) and protein sequences (e.g. PRABI_prodfit), as well as to view entries of cross-referenced database (e.g. PDB or GO). (C) PubMed entry of the article by Tsujimoto et al. reporting the discovery of the BCL-2 gene.

threshold is defined for use during the annotation process to classify the sequences into orthology groups (for BCL-2 homologous proteins) or clusters (for BH3-only proteins). The discovery mode is run periodically to improve the profiles sensitivity or when a new sequence is included in the seed set. The production mode is used to generate BCL2DB. The process starts by searching UniProtKB with the profile HMMs that were computed in the discovery mode. Then, for each selected sequence (E-value < 0.1) the Ensembl (10), Ensembl Genomes (11) or ENA entry is retrieved from UniProtKB cross-references or after a BLAST search. UniProtKB sequences corresponding to identical Ensembl or ENA entry are merged into one single entry. Unwanted annotations (i.e. uncertain, poor quality or nonconformity to the vocabulary standards) retrieved from Ensembl/ENA entries are then deleted to create a BCL2DB entry template that will be enriched with standardized data during the annotation procedure.

Annotation procedure

The annotation procedure (*AnnotateBCL2* program; Figure 1B) starts from the entry templates generated for sequences that belong to the group of BCL-2 homologs or

BH3-only proteins. The annotation process automatically affiliates each identified protein to its closest orthology group or cluster based on a specific curated gathering threshold cutoff (different for each profile). Above the threshold, the entry (typically a sequence from a nonmammalian organism) is considered as 'unclassified'. Moreover, homemade BH1-4 motif profiles were developed (see below) for use in computational annotation of BCL2DB sequences to precise the positions of their respective BH region(s). Finally, the Protein Data Bank (PDB) (12) sequences are searched for known structures with the profile HMMs.

BH motif annotation

We performed an *ab initio* motif discovery procedure by running the *meme* program of the MEME software suite (13) on a reference set of 158 amino acid sequences of BCL-2 homologous proteins and BH3-only proteins. Four position-specific scoring matrices corresponding to the four BH motifs were defined by *meme*. This original approach increases the sensitivity and specificity of BH-motif detection in protein sequences. The *mast* program uses the resulting position-specific scoring matrices to scan BCL2DB



Figure 3. Example of a protein sequence data set. **(A)** Partial view of the page giving access to cellular BCL-2 homologous protein data sets. The table lists available data sets for the diverse species and proteins in the BCL-2-like clade. The user can access sequences in Fasta/Pearson format (F letter), multiple sequence alignment in Clustal W format (C letter) and residue repertoire (R letter). **(B)** The Fasta/Pearson file for *Homo sapiens* BCL-2 protein sequences. The sequence identifiers are built with the primary accession number, the protein name and an isoform number. A link is provided on sequence identifier to view the BCL2DB entry (Figure 2B). **(C)** The multiple sequence alignment computed with MUSCLE and displayed in Clustal W format. The color code used is red, green, black for residues that are conserved, strongly similar, weakly similar and variable in the alignment column, respectively, as defined by Clustal W. Dashes indicate gaps. **(D)** Residue repertoire computed from the previous alignment with the same color code.

sequences for BH motifs. The *mast* results, which allow mapping of BH motifs onto BCL2DB sequences, are integrated in the BCL2DB entry as a protein sequence annotation.

Entry content

The text format of a BCL2DB entry is an extension of the ENA EMBL-Bank format (14, 15). The BCL2DB accession numbers (AC line, repeated in ID line) are the UniProtKB accession numbers of the sequences identified by the profile HMM searches. In a BCL2DB Pearson/Fasta file, the accession number is associated with the gene/protein name and an isoform number (if needed) to compose the sequence identifier. The description (DE line) and keyword (KW line) fields of a BCL2DB entry contain information about the classification and the BH motifs of the sequence,

as computed during the annotation procedure. The bibliographic references (RN, RC, RP, RA, RT and RL lines) are merged from the ENA and UniProtKB entries. Cross-references (DR lines and *db_xref* and *PRABI_prodf* qualifiers) to ENA, Ensembl, Ensembl Genomes, Gene Ontology (16), Human Protein Atlas (17), Protein Data Bank, RefSeq (18), NCBI Taxonomy (19) and UniProtKB are also provided. An additional cross-reference is provided to link each entry to the related BCL2DB reference sequence. The features (FT lines) retrieved from ENA, Ensembl and Ensembl Genomes entries are enriched in protein annotations through *PRABI_prodf* qualifiers under *mat_peptide* features. The *PRABI_prodf* qualifiers follow the feature table format of the UniProtKB database and describe information about proteins (e.g. chains, domains or sites). The protein annotation data added are the structural

Conclusion and perspectives

BCL2DB is a collection of computer-annotated BCL-2-related sequences. The automatic annotation process used to generate the BCL2DB entries guarantees updates of the data and standardized annotations. The latter allow efficient keyword searches useful to generate sequence data sets available through the Web interface. Sequences can be retrieved for further analysis with a set of bioinformatics tools available in the NPS@ server. The BCL2DB Web site also allows researchers to access up-to-date knowledge about BCL-2 family members and BH3-only proteins and to annotate their own sequences through the BCL2DB automatic annotation process. In its current implementation, BCL2DB offers a good template to integrate new annotation data that will enrich its content in the future (e.g. gene expression, interaction data, information on posttranslational modifications) and will enhance its Web site with new analysis tools (e.g. to identify novel BH3-only proteins and splice variants) and a search tool to perform dynamic queries on the database to extract data sets of interest to the user. BCL2DB can serve as a reference for the analysis of data generated by means of high-throughput technologies. We put a lot of attention and rigor in developing BCL2DB to provide a helpful and powerful tool to both 'BCL-2-ologists' and researchers working in the various fields of physiopathology.

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