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The evolution of BIR domain and its containing proteins

Lihuan Cao, Zhenghan Wang, Xianmei Yang, Li Xie, Long Yu*

State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Sciences, Fudan University, Shanghai 200433, PR China

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Abstract **BIR domain and its containing proteins play critical roles in cell apoptosis and cell division. Here several lines of novelty were revealed based on a comprehensive evolutionary analysis of BIR domains in 11 representative organisms. First, the type II BIR domains in Survivin and Bruce showed more conservation compared with the type I BIR domains in the inhibitors of apoptosis proteins (IAPs). Second, cIAP was derived from a XIAP duplicate and emerged just after the divergence of invertebrates and vertebrates. Third, the three BIR domains of NAIP displayed significantly elevated evolutionary rates compared with the BIR domains in other IAPs.**

Keywords: BIR domain; BIR domain containing protein; Evolution

1. Introduction

It is now widely accepted that domains constitute the basic structural, functional or evolutionary unit of proteins [1]. Understanding the evolution of single domains and domain architectures is of great importance to unravel the functions of proteins.

The BIR domain is a structurally distinct, zinc-finger fold domain [2]. BIR domains are found in viruses, yeasts and metazoans, but not in plants. Previously, BIR domains were suggested to be divided into two types based on their structural and functional differences [3,4]. The type I BIR domains were found in BIR domain containing proteins (BIRps), which encompass those that inhibit cell death (they are appropriately called inhibitor of apoptosis proteins, IAPs) [3,4]. The IAPs all contain type I BIR domains, and usually also possess a carboxy-terminal RING finger domain. The human genome has 6 genes that encode IAPs, and they are *XIAP, cIAP-1, cIAP2, NAIP, ML-IAP,* and *ILP-2*. The type II BIR domains were presented in BIRps which include mammalian Survivin/BIRC5 and Bruce/BIRC6, *Caenorhabditis elegans* BIR-1 and BIR-2, and *Drosophila melanogaster* proteins d-Bruce and Deterin [4]. Survivin and Bruce were demonstrated to play essential roles in cell division [5,6].

Given the important functions of BIR domain containing proteins, the evolutionary history of the BIR domain and BIRps has been studied previously [4]. Some studies on the apoptotic proteins set in representative organisms have also provided insights into the evolution of the BIR domain [7,8]. However, these studies mainly focused on the BIR domains from three or four organisms, and it was difficult to get a detailed map of the evolutionary history of BIR domains. Here, we collected the sequence data of BIR domains from 11 representative organisms, which include two fungi (*Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*), two protostomes (nematode (*Caenorhabditis elegans*) and fruit fly (*Drosophila melanogaster*)), two invertebrate deuterostomes (ascidian (*Ciona intestinalis*) and sea urchin (*Strongylocentrotus purpuratus*)), five vertebrates (human (*Homo sapiens*), mouse (*Mus musculus*), chicken (*Gallus gallus*), frog (*Xenopus tropicalis*) and zebrafish (*Danio rario*)), and employed comprehensive phylogenetic analysis. The sea urchin and the ascidian were selected because they occupy important evolutionary positions with respect to the transition from invertebrates to vertebrates. The zebrafish was selected as a representative basal vertebrate.

The evolutionary history of viral BIR domains was not covered in this study because we mainly focused on BIR domains from fungi and metazoans. It has been suggested that BIR domain containing proteins in baculovirus were pirated from the genomes of cells they infected [9], and a detailed phylogenetic analysis of IAP from insect viruses and their hosts confirmed that viral IAP genes arose by "gene capture" from their hosts [10].

In addition, proteins or protein domains may have different evolutionary rates, which would suggest that they are subject to different degrees of selection pressure [11]. Thus, we used three substitution rates (*Ka*, *Ks* and *Ka*/*Ks*) to compare the evolution of the BIR domains in mammalian lineages.

2. Materials and methods

2.1. Database Searches

Using the sequence of the BIR domain from human Survivin/BIRC5 as a query, PSI-Blast [12] and BLASTP searches were carried out at the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov) and at the Ensembl database (http://www.ensembl.org), and all sequence data of BIRps were collected. The sequences of BIRps from sea urchin were also collected by searching the BIR domain HGSC urchin annotation database (http://annotation.hgsc.bcm.tmc.edu/Urchin/cgi-bin/pubLogin.cgi).

2.2. Sequence alignments and phylogenetic analysis

The sequences of BIR domains from every protein were collected by analyzing every protein sequence with the protein domain prediction

*Corresponding author. Fax: +86 021 65643954.
E-mail address: longyu@fudan.edu.cn (L. Yu).

Abbreviations: NJ, neighbor-joining; BIR, BIR domain; BIRps, BIR domain containing proteins; IAPs, inhibitor of apoptosis proteins; Hs, *Homo sapiens*; Mm, *Mus musculus*; Gg, *Gallus gallus*; Xt, *Xenopus tropicalis*; Dr, *Danio rario*; Ci, *Ciona intestinalis*; Sp, *Strongylocentrotus purpuratus*; Ce, *Caenorhabditis elegans*; Dm, *Drosophila melanogaster*; Spo, *Schizosaccharomyces pombe; Sc, Saccharomyces cerevisiae*

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program SMART [13]. Preliminary multiple sequence alignments of the BIR domains were carried out by using MUSCLE program [14]. Phylogenetic trees were generated for BIR domain sequences using neighbor-joining methods in the PHYLIP (Version 3.63) program [15] (Bootstrap replicates were set as 1000). Bayesian analyses were carried out by MrBayes program [16] with default settings, while the MCMC search itself was continued for 1 000 000 generations, sampled every 100 generations, and 2500 trees were discarded as burnin. Tree files were viewed by using the MEGA program [17].

2.3. Evolution rate analysis

For calculation of mouse/human *Ka*/*Ks* ratios, amino acid sequences of BIR domains from orthologous BIRps were aligned, and the alignments obtained were transferred to the cDNA sequences by searching the GenBank database. The methods of Nei and Gojobori (1986) implemented in Paml package [18] were used for calculation.

3. Results and discussion

3.1. BIR domain containing protein sequences identification

BIR domain containing protein sequences were identified as described in the Section 2. Only protein sequences that contain almost the entire BIR domain were selected for analysis. Some retrieved sequences were discarded on the basis of the following criteria: (1) duplicated database submissions of the same sequence; (2) alternatively spliced isoforms. In total, we identified 115 BIR domains from 59 BIRps from 11 organisms (details of protein accession number, domain composition, and amino acid sequences can be found in Supplementary files 1 and 2). Mouse possesses several NAIP genes and the sequences of these genes are almost the same as each other, therefore only one representative mouse NAIP protein sequence was used for the analysis.

3.2. The general evolutionary history of BIR domain and proteins containing it in invertebrate and vertebrate

We tried to analyze the evolution of BIR domains from the 11 species from yeast to human in one step, but the results were not sound, mainly due to the complication of the evolutionary process of the BIR domain over its long history. Therefore, we divided our analysis into two steps.

To analyze the general evolution history of BIR domains in invertebrates and vertebrates, we performed neighbor-joining analysis based on the sequences of BIR domains from seven organisms (*S. pombe, S. cerevisiae, D. melanogaster, C. elegans, C. intestinalis, S. purpuratus* and *H. sapiens*) (Fig. 1). Similar results were obtained by Bayesian analysis [16] (Supplementary file 3).

Firstly, the type II BIR domains in Survivin and Bruce showed more conservation compared with the type I BIR domain in IAPs during the evolutionary process from invertebrate to vertebrates. The BIR domains in human Survivin, Ci-02205 (Survivin ortholog in *C. intestinalis*) and Sp-08878 (Survivin ortholog in *S. purpuratus*) were clustered in one branch (Survivin-group) with a high supporting bootstrap value, and the BIR domains in human Bruce, Sp-01262 (Bruce ortholog in *S. purpuratus*) and Dm-Bruce (Bruce ortholog in *D. melanogaster*) also were clustered in another branch (Bruce-group).

Survivin and Bruce are major regulators of cell division [5,6]. Previously, Dr. Silke suggested that the type II BIR domain in Survivin represents the earliest BIR domain, and following a gene-duplication event, the BIR domains in IAPs were evolved and gained a different function [3]. Our data suggested that the type II BIR domain showed more evolutionary conservation than the type I BIR domain in eukaryotes.

The type I BIR domains diversified greatly from invertebrates to vertebrates. It was found that some BIR domains in ascidian and sea urchin tended to cluster in several independent groups within species, such as the Ci-group-1, Ci-group-2 and Ci-group-3 for ascidian proteins, and the Sp-group for sea urchin proteins. This suggested that the increasing number of BIR domain containing proteins in invertebrate deuterostomes ascidian and sea urchin was generally achieved by species-specific duplication of BIR domains or BIRp genes.

3.3. Evolution of BIR domain and BIR containing proteins in vertebrates

To analyze the detailed evolutionary history of the BIR domain in vertebrates, we carried out a neighbor-joining analysis from BIR domain sequences from five vertebrates (*H. sapiens, M. musculus, G. gallus, X. laevis* and *D. rario*) (Fig. 2). Bayesian analysis [14] returned similar results (Supplementary file 4).

The human genome has six genes that encode IAPs (*XIAP, cIAP-1, cIAP2, NAIP, ML-IAP* and *ILP-2*). Human XIAP contains three BIR domains and a carboxy-terminal RING finger domain. The human cIAP-1 and cIAP-2 are very similar to each other and both proteins have three BIR domains, a caspase recruitment domain (CARD) and a RING finger. We found that the three BIR domains of human XIAP and its orthologs fall into three different subgroups (XIAP-BIR1-group, XIAP-BIR2-group and XIAP-BIR3-group), respectively. For some unknown reason, the BIR2 domain from Xt-XIAP (the XIAP protein from zebrafish) failed to locate in the XIAP-BIR2-group. The first BIR domains of human cIAP1, cIAP2, and their orthologs were clustered into the XIAP-BIR1-group. The second BIR domains of human cIAP1, cIAP2, and their orthologues were clustered into the XIAP-BIR2-group. The third BIR domain of human cIAP1, cIAP2, and their orthologs formed a cIAP-BIR3-group. However, the cIAP-BIR3-group was located close to the XIAP-BIR3-group. Given the generally similar subgroup classification of BIR domains from cIAPs and XIAP in the phylogenetic analysis, we speculated that cIAPs evolved from XIAP by the insertion of a CARD domain between the three BIR domains and the RING finger domain. In addition, the CARD containing IAPs have been identified only in vertebrates, implying that the insertion of CARD could be dated back to after the divergence of invertebrates and vertebrates.

Possibly due to the evolutionary relationship of XIAP and cIAPs, it was found that some functional similarity or redundancy still existed in these two kinds of molecules. It was reported that BIR domain 1 of XIAP could form a dimmer and activate NF-κB [19]. The BIR domain 1 of cIAP1 and cIAP2 have also been reported to be dimerized and mediate NF-κB signaling [20]. It has also been demonstrated that cIAPs and XIAP can bind pro-apoptotic molecules and target them for degradation by the ubiquitin-proteasome pathway [21,22]. The BIR domains 2 and 3 of XIAP are involved in blocking the active sites of caspase-3, -7 and -9 [23,24]. The BIR domains 2 and 3 of the two cIAPs are also able to bind caspases-7 and -9 but are only weak caspase inhibitors [24].

NAIP also contains three BIR domains, but their orthologs have only been detected in mammals. NAIP does not have a RING finger domain but has a nucleotide-binding NACHT

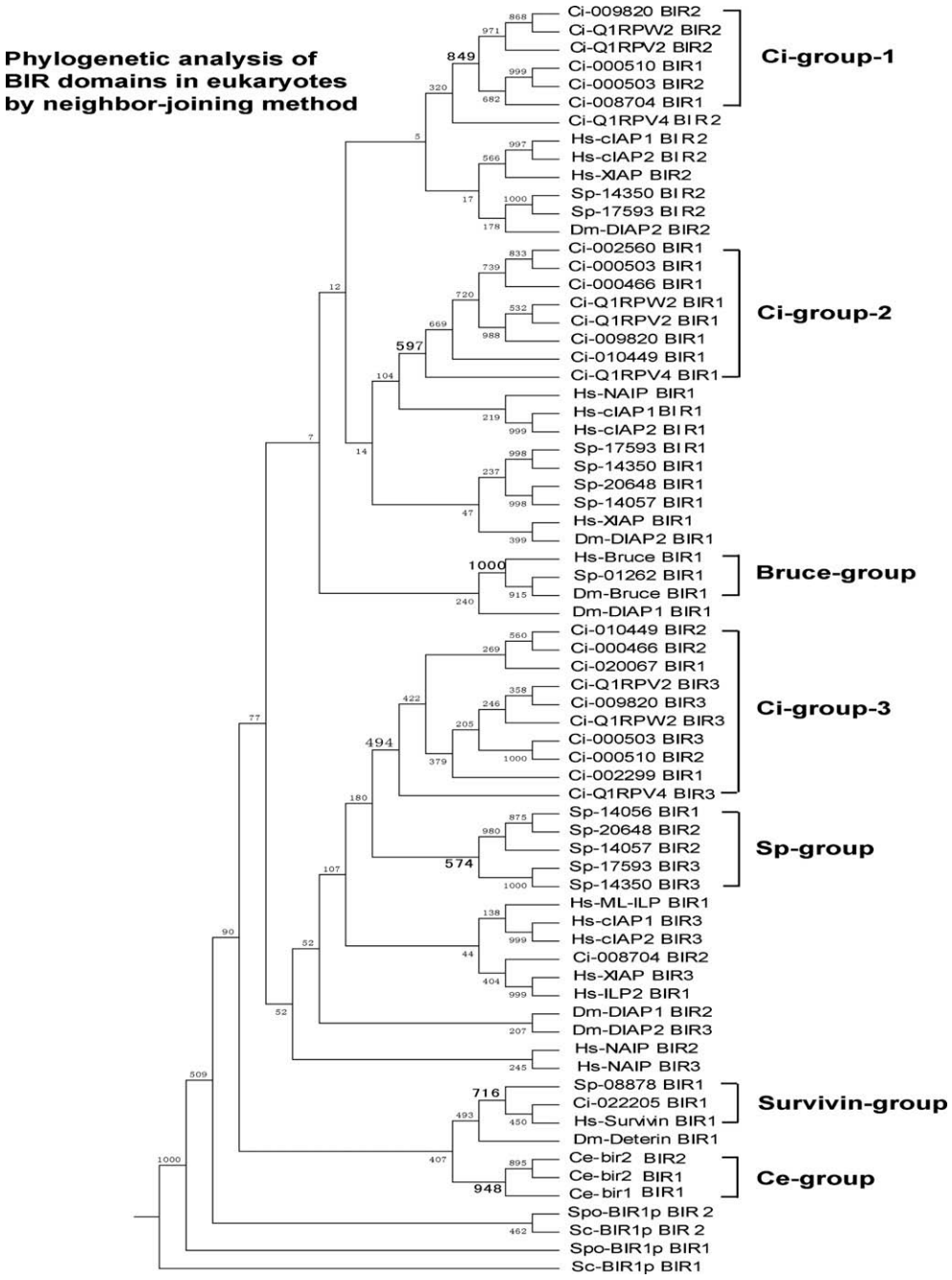

Fig. 1. Phylogenetic relationships of BIR domains from BIR domain containing proteins in eukaryotes. The trees shown were inferred by the neighbor-joining method. The values on the tree nodes are neighbor-joining bootstraps. The BIR domains are indicated by species name, protein name and BIR domain position, such as hs-XIAP BIR2 indicated the second BIR domain from human XIAP. The organism abbreviations used are listed below: Hs, *Homo sapiens*; Ci, *Ciona intestinalis*; Sp, *Strongylocentrotus purpuratus*; Ce, *Caenorhabditis elegans*; Dm, *Drosophila melanogaster;* Spo, *Schizosaccharomyces pombe,* Sc, *Saccharomyces cerevisiae*.

domain at the C-terminus. The three BIR domains of NAIP did not have the similar subgroup classification of the BIR domain from XIAP and cIAPs (Fig. 2). Due to this difference, it is unclear whether NAIP was also derived from XIAP by gene duplication similar to the cIAPs.

Another human IAP, ML-IAP/Livin, contains a single BIR domain and RING finger domain. The BIR domain of Livin did not fall into any branch of BIR domains from other human IAPs. ILP-2/BIRC8 is the most recently identified IAP in human and it has a BIR domain and a RING finger domain. The BIR domain of ILP-2 was located in the X-BIR3-subgroup, and this was consistent with a previous report that proposed that ILP-2 was a XIAP fragment and ILP-2 gene was generated by a retroprocessing event [25].

Based on the previous reports and our analysis, we have tried to postulate a possible scenario for the evolution of BIR domain containing proteins (Fig. 3). The representative BIR proteins with these representative domain architectures in some organisms can be found in Supplementary file 5.

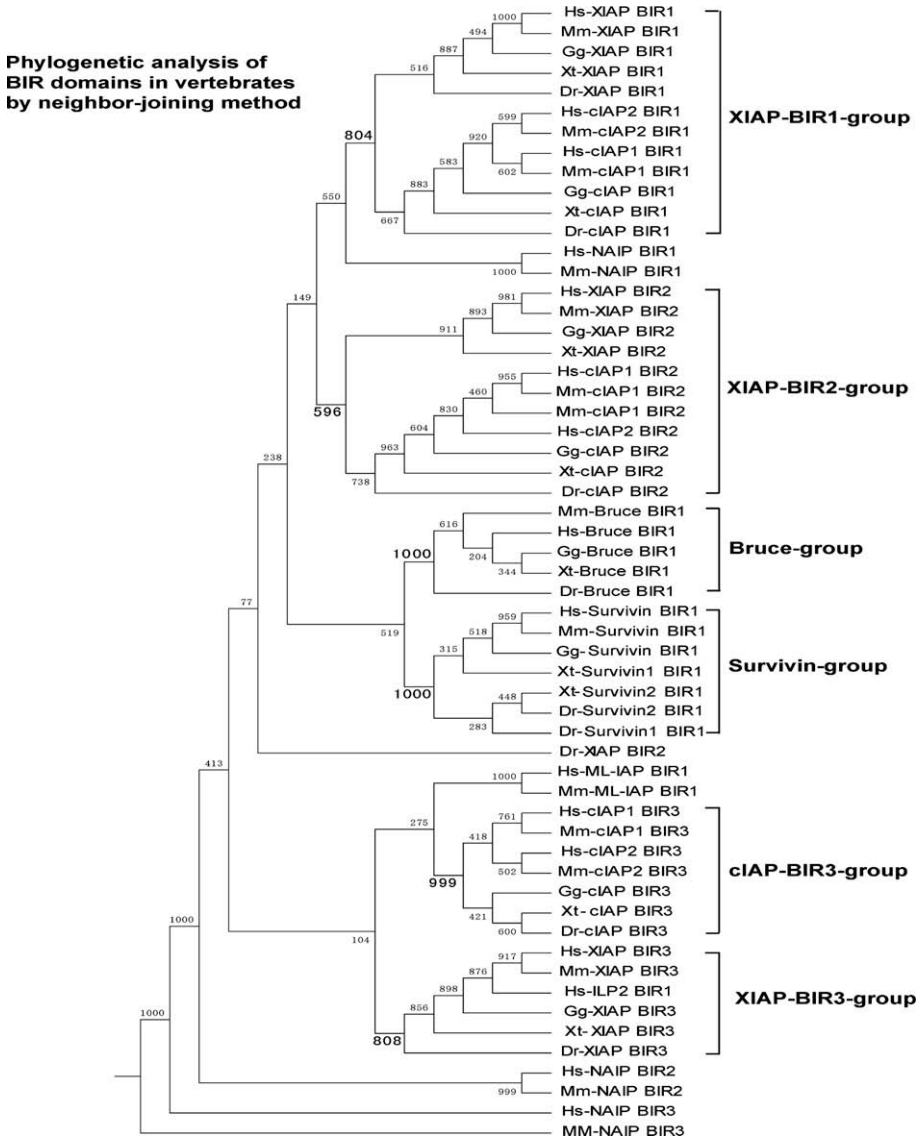

Fig. 2. Phylogenetic relationships of BIR domains from BIR domain containing proteins in vertebrates. The trees shown were inferred by the neighbor-joining method. The values on the tree nodes are neighbor-joining bootstraps. The BIR domains were indicated by species name, protein name and BIR domain position, such as Hs-XIAP BIR2 indicating the second BIR domain from human XIAP. The organism abbreviations used are listed below: Hs, *Homo sapiens*; Mm, Mus musculus; Gg, *Gallus gallus*; Xt, *Xenopus tropicalis*; Dr, *Danio rario.*

3.4. Different selective pressures for different BIR domains

For protein coding sequences, the synonymous rate (*Ks*) is often regarded as a measure of the underlying mutation rate, though it may be influenced by other factors. By contrast, the non-synonymous rate (*Ka*) or the ratio *Ka*/*Ks* is regarded either as a measure of the amount of purifying selection or positive selection.

We investigated the evolutionary rate of BIR domains in mammalian lineages (Table 1). We found that the *Ka* value and *Ka*/*Ks* value of BIR domain in Bruce is extremely low, indicating it evolved very slowly and was under very high selective purification pressure. Interestingly, the *Ka*, *Ka*/*Ks* values for the BIR domains of NAIP are much higher than the BIR domains from other BIRps, and this indicated that BIR domain from NAIP displayed dramatically elevated evolutionary rates compared with other BIRps. As we noted, the three BIR domains from NAIP also showed clear differences in the phylogenetic tree compared with the BIR domains from XIAP and cIAPs (Section 3.3).

NAIP was also reported to have a role in suppression of apoptosis [26]. However Eckelman et al. thought that the drastic substitution of Lys 143 of NAIP BIR2 domain replacing the equivalent Asp 148 of XIAP would preclude the inhibition of caspases, and the third BIR domain of NAIP might not even contain an IBM-interacting groove based on the sequence-conservation arguments [24]. Our phylogenetic and evolutionary rate analyses suggested that the BIR domains of NAIP might be under altered evolutionary or functional constraints compared with the BIR domains in XIAP/cIAPs. We tend to agree with Eckelman et al. and speculate that the BIR domains of NAIP do not share the function of inhibiting caspases with XIAP. Whether the BIR domains from NAIP have gained a different function and the detailed function of NAIP remain to be investigated by further research.

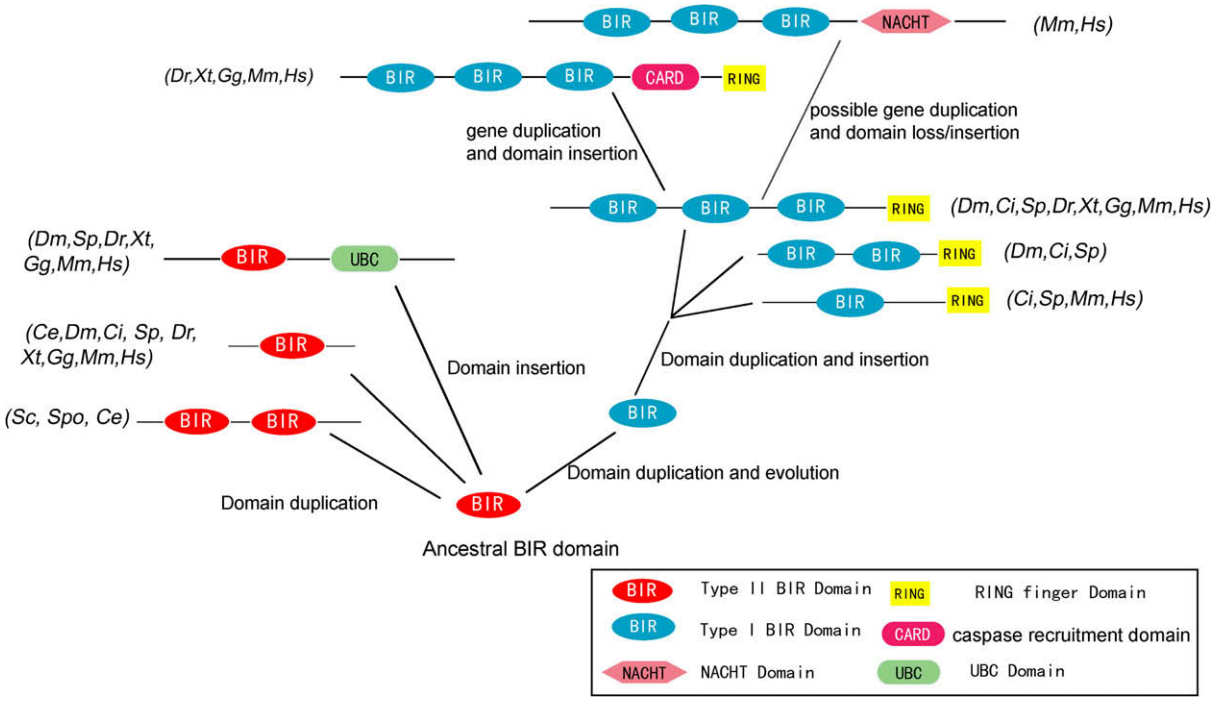

Fig. 3. Proposed scenario for the evolution of BIR domain containing proteins. The possible evolutionary map of BIR domain containing proteins with representative domain architectures was postulated. The lengths of protein domains and the whole protein are not shown on scale. The organism names that possessed the representative domain architecture proteins are also listed. Abbreviations: Hs, *Homo sapiens*; Mm, Mus musculus; Gg, *Gallus gallus*; Xt, *Xenopus tropicalis*; Dr, *Danio rario*; Ci, *Ciona intestinalis*; Sp, *Strongylocentrotus purpuratus*; Ce, *Caenorhabditis elegans*; Dm, *Drosophila melanogaster;* Spo, *Schizosaccharomyces pombe*, Sc, *Saccharomyces cerevisiae.*

Table 1
Synonymous (*Ks*) and non-synonymous (*Ka*) nucleotide substitution rates for BIR domains of human and mouse BIRps.

	Type II		Type I												
	Survivin	Bruce	XIAP			cIAP1			cIAP2			NAIP ML-ILP			ML-ILP
	BIR1	BIR1	BIR1	BIR2	BIR3	BIR1	BIR2	BIR3	BIR1	BIR2	BIR3	BIR1	BIR2	BIR3	BIR1
Ks	0.0456	0.0000	0.0310	0.0239	0.0312	0.0642	0.0307	0.0424	0.0778	0.1139	0.0817	0.1948	0.0828	0.1857	0.0887
Ka	0.8485	0.6310	0.4345	0.5116	0.3490	0.5916	0.7396	0.4242	0.8969	0.8104	0.5468	0.6839	0.6074	0.5723	0.8174
Ka/*Ks*	0.0538	0.0000	0.0714	0.0467	0.0895	0.1085	0.0415	0.1000	0.0867	0.1406	0.1494	0.2848	0.1364	0.3244	0.1085

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2008.09.058.

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