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Comparative Study of Apoptosis-related Gene Loci in Human, Mouse and Rat Genomes

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Abstract Many genes are involved in mammalian cell apoptosis pathway. These apoptosis genes often contain characteristic functional domains, and can be classified into at least 15 functional groups, according to previous reports. Using an integrated bioinformatics platform for motif or domain search from three public mammalian proteomes (International Protein Index database for human, mouse, and rat), we systematically cataloged all of the proteins involved in mammalian apoptosis pathway. By localizing those proteins onto the genomes, we obtained a gene locus centric apoptosis gene catalog for human, mouse and rat. Further phylogenetic analysis showed that most of the apoptosis related gene loci are conserved among these three mammals. Interestingly, about one-third of apoptosis gene loci form gene clusters on mammal chromosomes, and exist in the three species, which indicated that mammalian apoptosis gene orders are also conserved. In addition, some tandem duplicated gene loci were revealed by comparing gene loci clusters in the three species. All data produced in this work were stored in a relational database and may be viewed at http://pcas.cbi.pku.edu.cn/database/apd.php.

Key words apoptosis; gene cluster; comparative genomics; bioinformatics

Apoptosis is a regulated program by which cells destroy themselves [1]. The core proteins of the apoptosis machinery often include some principal apoptosis domains, and some of these domains not only exist in animals and plants, but also in unicellular eukaryotes and even in bacteria [2–4]. Recently, Doctor *et al*. collected all apoptosis related proteins from the NCBI nr database based on a domain search strategy and developed the apoptosis database (ADB, http://www.apoptosis-db.org/) [5]. According to the expressed mouse cDNA data from the RIKEN project, Reed *et al*. reported that there are a similar number of apoptosis and inflammation genes in mouse as in human; by comparing over 15 protein family groups between mouse and human, they revealed that most apoptosis genes in the two species arose early in mammalian evolution, but some other genes were amplified recently after human and mouse diverged and were represented as tandem copies on chromosomes [6]. However neither the ADB nor the comparative study took advantage of the three available mammal genomes, therefore systematic study of apoptosis related genes taking account of their chromosomal locations, especially comparative study of the apoptosis gene clusters among mammals, has not yet been done.

In this study, using the International Protein Index database (IPI [7], containing the public draft proteomes for human, mouse and rat), we combined the MotifCentric annotation system (MCAS, developed locally) and EBI InterProScan annotation for these proteins to catalog all apoptosis related proteins in human, mouse and rat, then mapped the identified apoptotic proteins onto their respective genomes. Thus we provided a gene loci centric

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view of the mammal apoptosis machinery. We further conducted phylogenetic analysis, and made the comparative gene loci clusters analysis among the three species based on the chromosomal localization of apoptosis related genes.

Materials and Methods

Data sources

Human IPI 2.20, mouse IPI 1.13 and rat IPI 1.3 were downloaded from the EBI IPI website (http://www.ebi.ac.uk/IPI/IPIhelp.html) and were used to catalog apoptosis related proteins. Genome sequence data (human build hg15, mouse build mm3, and rat build rn3) were from the GoldenPath website (http://genome.ucsc.edu/).

Mining IPI proteomes for apoptosis related proteins

We collected keywords about apoptosis related domains from published work [3,4,6], and submitted them to search the InterPro [8] database (version 7.0). Each of the resulting 21 InterPro entries represented one protein domain or motif, but they only corresponded to 16 apoptosis related protein families (**Table 1**).

Previously we reported a ProteinCentric Annotation System (PCAS) which employs a collection of motif and domain based algorithms to annotate model proteomes [9]. In this system, we used InterPro to cross reference various motif or domain databases and integrate the annotation results generated by different algorithms. The pre-computed annotation data in PCAS can also be tuned to allow for motif or domain based protein family queries. Using the same backend pre-computed annotation databases, one can query for all the proteins which contain a certain domain computed by any algorithm in PCAS. We also developed a web query system which allows non-redundant display of domain hits, and termed it MotifCentric Annotation System (MCAS). Given an InterPro ID, MCAS will return all the proteins containing the domain or motif represented by this InterPro ID. Thus for each of the three species, taking the above 21 InterPro

Table 1 Apoptosis related InterPro domains/motifs

Index	InterPro AC	InterPro domain/motif name	Functional roles in apoptosis pathway *
1	IPR006052	TNF (tumor necrosis factor)	Ligand
2	IPR001368	TNFR (TNF receptor)	Receptor
3	IPR000488	DD (death domain)	Receptor, adaptor, kinase
4	IPR001875	DED (death effector domain)	Adaptor, caspase
5	IPR001315	CARD (caspase recruitment domain)	Adaptor, kinase, caspase, caspase inhibitor
6	IPR004020	PYRIN	Adaptor
7	IPR000157	TIR (toll-interleukin-receptor domain)	Receptor, adaptor, Ap-ATPase
8	IPR002083	MATH (merphin and the TRAF-homology domain)	Adaptor
9	IPR001370	BIR (baculovirus inhibitor repeat)	Caspase inhibitor
10	IPR002475	Bcl-2 like (Bcl2-like apoptosis inhibitor)	Bcl2 family protein
	IPR000712	Bcl2_BH (apoptosis regulator Bcl-2 protein, BH)	Bcl2 family protein
	IPR003093	Bcl2_BH4 (apoptosis regulator Bcl-2 protein, BH4)	Bcl2 family protein
	IPR004725	Bcl-X (apoptosis regulator Bcl-X protein)	Bcl2 family protein
11	IPR002182	NB-ARC domain (Ap-ATPase domain)	Ap-ATPase
12	IPR007111	NACHT NTPase domain	NACHT nucleoside triphosphatase
13	IPR001309	ICE_p20 (caspase large subunit)	Caspase
	IPR002138	ICE_p10 (caspase small subunit)	Caspase
	IPR002398	ICE (caspase precursor)	Caspase
14	IPR000451	NFκB	Transcription factor
15	IPR003103	BAG	
16	IPR003508	CAD (caspase activated DNase)	Caspase substrate

* information was collected from published work [3,4,6] and the InterPro database.

entries as query, we obtained 21 groups of candidate apoptosis related proteins.

In addition, in the Swiss-Prot format files of the downloaded IPI proteomes, each IPI protein was linked to various databases, such as GO, HUGO/MGI/RGD, LocusLink and InterPro. The InterPro cross reference was obtained by InterProScan pre-computation. For each species, we implemented a perl script to parse the IPI Swiss-Prot format file and extract IPI proteins that are linked to the 21 InterPro IDs, and obtained another 21 groups of candidate apoptosis related proteins. We also extracted the HUGO/MGI/RGD gene names for the resulting IPI proteins.

Furthermore, we combined the two sets of protein groups, then manually checked those proteins that bear weak apoptosis domain signals ($E \geq 1e{-}5$) to eliminate false positives.

Mapping apoptotic proteins to the genomes and generating gene loci centric datasets

We ran the BLAT program [10] with default parameters to localize the apoptosis related IPI proteins to the corresponding genome sequences. For the human, we parsed the BLAT results using cutoffs: length coverage≥0.95, alignment identity≥0.95; for the mouse and rat, we used released cutoffs: length coverage≥0.9, alignment identity≥0.95, because the genomes of these two species are not very well sequenced and assembled. Under these cutoffs, five human, thirteen mouse and nine rat IPI proteins were not mapped, but we manually checked them using TBLASTN search at the NCBI website and BLAT search at the GoldenPath website. Three rat IPI proteins were not mapped and were excluded in the subsequent analysis. If several IPI proteins were localized to overlapped genomic regions in the same strand of a chromosome, which indicates that they are redundant sequences or the alternatively spliced protein isoforms of the same gene, then we would take the left-most and right-most coordinates of the overlapped genomic regions as the boundary of the gene locus.

Defining the homologous relationships among the three mammalian apoptosis gene loci sets

Since many apoptotic proteins are multi-domain proteins [6], for each of the 21 groups of proteins we combined proteins from the three species and ran the partial order alignment (POA) program [11] to perform multiple sequence alignment (MSA). The POA program is local alignment based and for multi-domain proteins it can produce more reasonable MSA than the global alignment based ClustalW program [12]. Taking each of the 21 MSAs as input, we went on to run Phylip to build a neighbor-joining tree for each group of proteins with the bootstrap test of 100 replications. Finally, we manually extracted the orthologous and paralogous relationships of the apoptosis proteins from each tree by referring to the bootstrap values on every node and the gene names tagged to the proteins. Taking the proteins as bridges, the apoptosis gene loci homologous relationships were obtained.

Results

Apoptosis related proteins and gene loci in the three mammals

In this study we cataloged 442 human, 325 mouse and 226 rat apoptosis related proteins from IPI proteome data, as described in "Materials and Methods". These proteins were further mapped to 232 human, 214 mouse and 155 rat gene loci on the corresponding genomes (**Table 2**). Phylogenetic analysis indicates that 144 (93%) of the rat gene loci have their orthologous counterparts in both human and mouse, and 190 (89%) of the mouse gene loci have their orthologous counterparts in human, which is a little lower than the result reported by Reed *et al*. (96%) [6]. Because IPI is a non-redundant proteome dataset [7], the inconsistency of protein number and gene loci number indicated that, on average, one mammalian apoptosis gene has 1.5–2 alternatively spliced protein isoforms.

In addition, for every species, **Table 2** lists the protein number and gene loci number in each domain group, and the detailed orthologous relationships may be viewed at our website. From **Table 2**, we can see that, for TNFR, CARD, PYRIN, Caspase (ICE) and NACHT domain groups, humans have more gene loci than mice, which is consistent with the results reported by others [6]. In addition, compared with previous results, we found more gene loci in terms of DD, CARD, PYRIN, Caspase (ICE), NACHT NTPase, TIR and NF-κB domain groups.

(1) For death domain, we detected OPG (IPI00298362) and UNC5CL (IPI00216869) genes bearing one death domain and conserved in three species. We also found several novel weak death domain signal bearing gene loci, some of which contain ankyrin motif.

(2) For TIR domain, we found one mouse gene locus (IPI00114413) which may code a novel TLR protein. Although we did not find its rat counterpart in current IPI data, we did find one rat protein in the NCBI nr database that shares very high protein identity (91%) with the mouse

Table 2 Apoptosis related IPI proteins and gene loci in human, mouse and rat

Domain/motif	InterPro AC	Human		Mouse		Rat	
		Protein #	Loci #	Protein #	Loci #	Protein #	Loci #
TNF	IPR006052	27	17	25	17	19	14
TNFR	IPR001368	49	28	39	26	21	16
DD	IPR000488	68	36	62	36	51	31
DED	IPR001875	35	7	9	7	5	5
CARD	IPR001315	58	30	31	24	28	19
PYRIN	IPR004020	40	22	20	15	8	6
Bcl-2 like	IPR002475	26	12	23	12	20	13
Bcl2_BH4	IPR003093	6	3	9	3	7	4
Bcl2_BH	IPR000712	34	16	27	16	24	16
Bcl-X	IPR004725	22	10	19	9	18	11
ICE	IPR002398	53	19	16	12	20	13
ICE_p20	IPR001309	53	19	16	12	20	14
ICE_p10	IPR002138	29	14	11	10	12	12
MATH	IPR002083	25	14	23	17	13	9
NACHT NTPase	IPR007111	45	25	40	23	15	11
NB-ARC	IPR002182	5	1	4	2	2	2
TIR	IPR000157	43	27	26	24	22	19
NF-κB	IPR000451	31	10	24	10	17	10
BIR	IPR001370	13	9	17	8	11	6
BAG	IPR003103	11	8	7	5	5	4
CAD	IPR003508	12	5	7	5	6	5
Total		442	232	325	214	226 *	155

Because some of the apoptosis proteins have multiple apoptotic domains, they were included in each domain group and calculated multiple times. Therefore, the total protein number and gene number are not equal to the sum of protein and gene numbers in all domain groups. * three rat IPI proteins were not mapped to the rat genome (see "Materials and Methods").

IPI protein. We found the mouse TRIF gene (IPI00170381), the SARM (IPI00007919) gene in the three species having TIR domain, and we included some interleukin receptor loci which were excluded by Reed *et al.*.
(3) For NF-κB2, we found two gene loci (IPI00196187, IPI00197774) in rat, and they share 81% protein identity.
(4) For CARD, PYRIN, ICE and NACHT domain groups, we identified many paralogous loci which form gene loci clusters on chromosomes.

Apoptosis gene loci clusters

Fig. 1 shows the chromosomal distribution of human apoptosis related gene loci. We found that, excluding chromosome 21 and Y, human apoptosis gene loci were not proportionally distributed on all other chromosomes. Of the 22 chromosomes, chromosome 1, 11 and 19 have the most gene loci, and in some certain chromosomal regions, apoptosis gene loci are obviously clustered. This could also be seen in mouse and rat's chromosomal distribution maps (available on our website).

To study the apoptosis gene loci clusters in the three species, we defined a cluster as two or more apoptosis genes separated by a distance less than 0.5 Mb, ignoring whether other genes are present [13]. Under this criteria, we observed 32 human gene loci clusters which include at least two gene loci. These 32 loci clusters in total include 98 (42%) human apoptosis gene loci, and the median and mean distances of the neighboring gene loci are about 29 kb and 100 kb. Similarly, 79 (37%, median: about 58 kb, mean: about 114 kb) mouse and 41 (26%, median: about 49 kb, mean: about 117 kb) rat gene loci respectively form 32 and 18 gene loci clusters that include at least two loci.

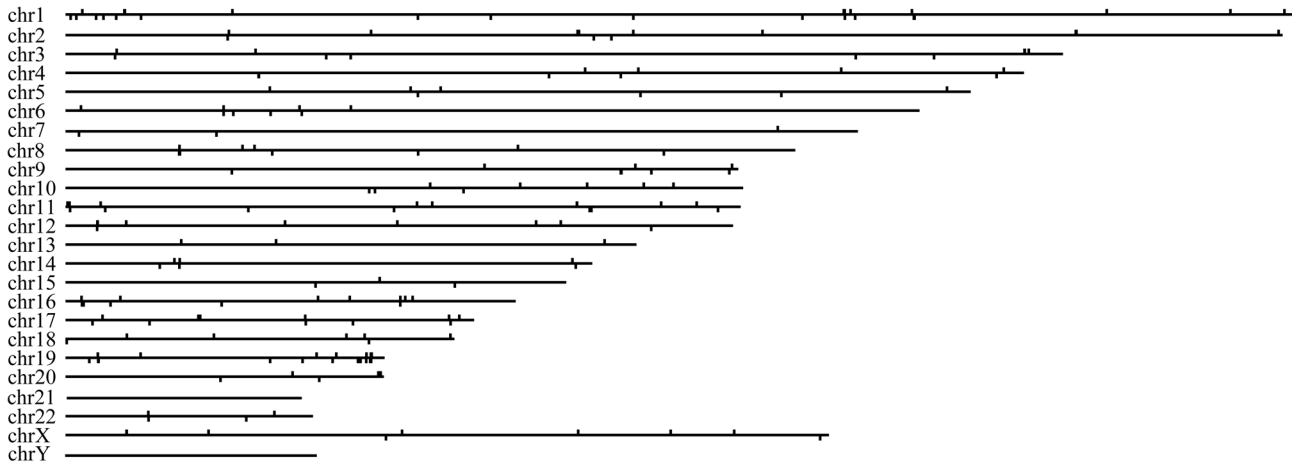

Fig. 1 Distribution of apoptosis related gene loci on human chromosomes

The horizontal lines represent the chromosomes. Vertical bars above and below the lines represent the locations of apoptosis gene loci.

Since the mouse's and rat's proteome and genome data are not as complete as the human's, we conclude here that about one-third of apoptosis gene loci in mammals are neighboring physically on chromosomes.

Table 3 summarizes the conservation of apoptosis gene loci clusters (orthologous loci in the same order) in the three mammalian species. This reveals that many apoptosis gene loci are not only conserved at individual gene level, but the gene orders are also conserved among the three species. The gene loci homologous relationships were obtained by phylogenetic analysis as described in "Materials and Methods". All of the human, mouse and rat apoptosis gene loci clusters mentioned above were included. To make the table more complete, some single gene loci were also added.

Table 3 shows two types of apoptosis gene clusters. The first type of gene cluster includes genes from the same gene family, and most of the conserved gene clusters in the table belong to this type. Genes in these clusters very likely derive from a common ancestor by early gene duplication and thus are paralogs in one species. Since most of the paralogous genes in **Table 3** are present in the three species, these duplications clearly happened before the speciation of the three mammals. Nevertheless we observed in some of the conserved gene clusters that some of the paralogous genes are only present in one or two species, such as the conserved gene cluster 1, 2, 5, 6, 8, 9 and 11. These reflect some recent gene duplication in human or mouse, which is likely to correlate with the different complexity of the apoptosis machinery in the three species. In **Table 3**, the other type of gene cluster includes genes from more than one gene family, and it is interesting to test whether these neighboring genes are expressed correlatively [14].

Taking the conserved loci cluster 1 (caspase-1,4,5,12, COP, ICEBERG) as an example, from **Table 3** and **Fig. 2**, we can see that there are 10 gene loci in the human genome, but only three in the mouse and rat genomes. In the three species, the gene order and orientation of three gene loci (caspase-1,4,12) are the same, but in human the caspase-1 gene seems to be distinctly amplified (11.15–21, for the locus nomenclature, please see the legend of **Table 3**). which confirmed and extended other reports [6,15]. It is worth mentioning that other than 11.15 and 11.18, all other gene loci either contain only ICE domain or only CARD domain, and at least some of them were reported to be the competitive inhibitors of functional caspases [6]. In addition, the caspase-4 gene family has two copies (CASP4, 5) in human, and the two paralogous genes are more similar to each other than to their mouse and rat counterparts, which indicates that there was a recent gene duplication in human after primates and rodents diverged.

As well as the above example, some other loci clusters have been reported, such as the NALP gene cluster in human and mouse [16], NAIP gene cluster in mouse [17], and IL1 gene cluster in human [18]. However, in our study, we extended the previous results and revealed that these clusters were conserved in the three mammals. All of the gene loci clusters in **Table 3** may be viewed at our website.

Discussion

In this study, we cataloged 232 human and 214 mouse

Table 3 Summary of conserved gene loci clusters in human, mouse and rat

Index	Gene Names	Human		Mouse		Rat	
		Loci clusters *	Numbers	Loci clusters *	Numbers	Loci clusters *	Numbers
1	Caspase-1,4,5,12, COP, ICEBERG	11.12–11.21	10	9.1–9.3	3	8.1–8.3	3
2	PYPAF-3,4,6, MATER, NALP-2,8,9,13,14	19.13–19.20, 11.4, 3.10	10	7.1,7.2–7.3,7.5, 7.7–7.8,7.12,13.6	8	N/A	N/A
3	IL1R1, IL1RL-1,2, IL18R1, IL18RAP	2.4–2.8	5	1.2–1.6	5	9.4–9.7	4
4	BIRC/NAIP	5.2	1	13.8–13.9	2	2.1	1
5	IFI16, MNDA, AIM2	1.16–1.20	5	1.18–1.25	8	13.7	1
6	TNFRSF10-A,B,C,D	8.1–8.4	4	14.7	1	N/A	N/A
7	TNFSF-1,2,3	6.2–6.4	3	17.2–17.4	3	20.1–20.3	3
8	TLR-1,6,10	4.1–4.3	3	5.1–5.2	2	14.1–14.2	2
9	CFLAR, caspase-8,10	2.13–2.15	3	1.7–1.8	2	9.8–9.9	2
10	TNFSF-7,9,14	19.2–19.4	3	17.9–17.11	3	UN.2	1
11	Caspase-14, PYRIN, NOD	16.1–16.3	3	16.1–16.2	2	10.3	1
12	TNFR1, LTBR, TNFRSF7	12.1–12.3	3	6.7–6.9	3	4.6–4.7	2
13	PIDD, SIGIRR, PYPAF5	11.1–11.3	3	7.16–7.18	3	1.10–1.12	3
14	SOB	N/A	N/A	7.19–7.21	3	N/A	N/A
15	TEF4-8	N/A	N/A	3.4–3.7 *	3	N/A	N/A
16	A1-a,b,c,d	15.3	1	Un_Random1–2	2	8.10	1
17	TNFSF-4,6,18	1.23–1.25	3	1.13–1.15	3	13.3–13.4	2
18	TLR-7,8	X.1–X.2	2	X.8–X.9	2	X.2	1
19	TNFSF-8,15	9.3–9.4	2	4.3–4.4	2	5.4	1
20	MEP1A, TNFRSF21	6.7–6.8	2	17.5–17.6	2	9.1–9.2	2
21	IL1RAP	3.8–3.9	2	16.5–16.6	2	11.1–11.2	2
22	CIDE-3, IRAK2	3.1–3.2	2	6.3–6.4	2	4.3	1
23	Bcl-Rambo, BID	22.1–22.2	2	6.5–6.6	2	4.4–4.5	2
24	BIRC7, TNFRSF6B	20.4–20.5	2	2.10	1	N/A	N/A
25	CLAN, BIRC6	2.1–2.2	2	17.12–17.13	2	6.1–6.2	2

The gene names were obtained as described in "Materials and Methods". * the number before the dot represents the chromosome that the gene was located on; the number after the dot is the index of the gene locus after we sorted all the apoptosis gene loci on one chromosome; gene loci were linked with a dash (–) to represent that they form a gene loci cluster. The IPI proteins that correspond to these gene loci may be obtained at our website. N/A, not available.

apoptosis related gene loci. Based on phylogenetic analysis, we proved that about 90% rodent apoptosis related genes have orthologous counterparts in human. These results are largely consistent with previous reports [6]. However we identified some new apoptosis proteins containing, for example, DD, TIR and NF-κB domains, because we used more comprehensive IPI proteomes as data sources and combined both the MCAS and InterProScan annotations which are based on the InterPro protein domain/motif database. We also identified 155 rat apoptosis gene loci, representing an estimated 70% of rat apoptosis gene repertoire.

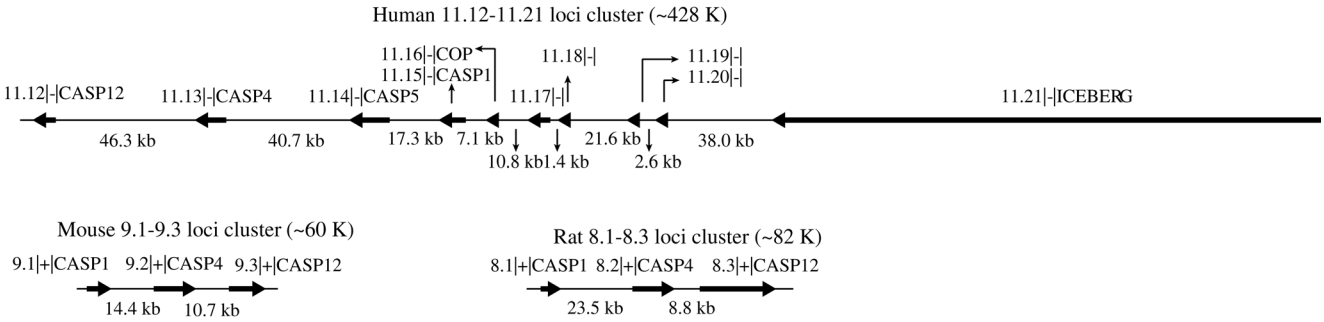

Fig. 2 Conserved caspase-1,4,5,12, COP, ICEBERG loci cluster in human, mouse and rat

The arrows represent the gene loci and their orientations. For each gene locus, we separated the gene locus name (see **Table 3** legend), chromosomal strand and gene name with "|". The distances between each neighboring gene loci are indicated.

To our knowledge, this study is the first comprehensive analysis of apoptosis gene loci clusters in mammals. We defined a distance criterion to determine which apoptosis genes are clustered, and observed that about one-third of mammalian apoptosis related genes neighbor each other. Although some previous studies reported the gene clustering cases in human or mouse [15–18], our analysis discovered all the gene loci clusters and found most of them are conserved in the three mammals. At the same time, comparing the loci clusters among the three species revealed some species-specific gene amplification or gene loss, which indicated some genes were tandem duplicated and were relative to some species-specific functions.

In fact, besides the classic caspase-dependent apoptosis pathway that we discussed in this paper, there are some caspase-independent cell death routes [19–23]. The existence of multiple death pathways is thought to be a safe mechanism to remove useless cells. Cells dying of these caspase-independent routes have different morphological alterations in their nucleus compared with those dying of classic apoptosis, and at the molecular level have some important genes involved such as *AIF*, *EndoG*, *HtrA* family genes, *DIABLO*/*Smac*, *A20* family genes and the genes of some cysteine and serine proteases and their inhibitors (including calpains, calpastatins, cyscathepsins, cystatins, serprots, serpins, granzymes, and cathepsins). In fact, so far only some of the above proteases were found to be related to cell death. Interestingly some previous work on *AIF* and *HtrA* genes revealed that very likely the caspase-independent cell death pathways are more ancient than the apoptosis pathway [2,22]. We extracted these genes by keyword searching the Gene database via NCBI Entrez web service and added these genes to our apoptosis related gene catalogs. Most of these genes were also found to be conserved in the three species.

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