

# Caspases

## Key References

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## Overview

Caspases are a family of cysteine proteases that cleave after an aspartic acid residue. The enzymes show significant homology with the cell death protein Ced 3 from the nematode worm *Caenorhabditis elegans*. Caspase activation appears to be an essential component of the cellular pathway for programmed cell death or apoptosis. All of the enzymes are synthesized as "proenzymes" which are biologically inactive. The proenzyme consists of a short or long prodomain together with one large (~20 kDa) and one small (~10 kDa) subunit. The active site of the enzyme contains an invariant cysteine in the sequence QACXG (where X is R, Q or G) in the larger subunit, while the smaller subunit determines substrate specificity. An active enzyme is formed by a heterotetramer of two small and two large subunits.

At the present time, there are 14 members of the caspase family, referred to as caspase 1-14, which may be subdivided in various ways. Phylogenetically, caspases 1, 4, 5, 11, 12 and 14 all belong to the caspase 1 subfamily. Caspase 2 is closest in sequence to caspase 9, but is grouped alone. Caspases 3, 6, 7, 8, 9 and 10 comprise the caspase 3 subfamily. Alternate classifications are based on whether the caspase has a long or short prodomain, or on substrate specificity. Most of the caspase 1 subfamily have long prodomains and are believed to play a role in inflammation. Within the caspase 2 and 3 subfamilies, a long prodomain will generally contain specific protein interaction motifs that interact with similar motifs in other proteins. Thus, the prodomain of caspases 8 and 10 contain DED (Death Effector Domain) regions and interact with death adaptor molecules containing DEDs such as FADD (Fas Associated Death Domain).

Several other caspases contain CARD (Caspase Recruitment Domain) regions in their prodomain resulting in aggregation with, and activation of, other procaspases. Caspases with CARD sequences in their prodomain aggregate via an interaction with the molecule apoptosis protease activating factor (APAF-1). Caspases that either bind and activate other caspases or are recruited by death adaptor molecules to signal a cascade of caspase activation are termed 'initiator caspases'. The initiator caspases are thought to act upstream of the short prodomain caspases or 'executioner caspases'. The short domain caspases are not directly regulated, but once activated cleave key intracellular substrates triggering the apoptotic collapse of the cell.

Caspases can also be divided into three groups according to their substrate and inhibitor profile. Group I caspases have a consensus sequence WEHD within their preferred substrates. The type of substrates cleaved include pro-interleukin-1 $\beta$  and interferon  $\gamma$ -inducing factor, suggesting a role in formation of mature pro-inflammatory cytokines, with little evidence for any involvement in a pro-apoptotic pathway. Group II caspases prefer an invariant aspartate at the start of their cleaved tetrapeptide. These caspases, which include the 'executioner' caspases, cleave many apoptotic substrates, i.e. proteins whose cleavage would be predicted to prevent DNA replication and repair and cause collapse of the cytoskeleton. Group III caspases cleave and activate many other pro-caspases as well as other 'apoptotic' substrates, implicating these enzymes as upstream activator caspases.

Many naturally occurring inhibitors of apoptosis, derived from mammalian, viral and bacterial sources, are now known to be caspase inhibitors. They include the Inhibitors of Apoptosis (IAP) family (mammalian) which bind to procaspases to prevent their activation. In addition, there are direct inhibitors, such as CrmA (viral) and p35 (baculovirus), that have different efficacy for individual caspases. There are also recently discovered indirect regulators of apoptosis, DIABLO/SMAC, which themselves bind to the IAPs and hence sensitize cells to apoptosis. Members of the BCL-2 family can be anti- or pro-apoptotic; they control the release of cytochrome C and other mitochondrial proteins and hence affect the binding of APAF-1 to the initiator caspase 9 and the resultant activation of the executioner caspase, caspase 3.

In addition to these naturally occurring inhibitors of caspase activity, there are many synthetic substrates and inhibitors. Most are based on tetrapeptides corresponding to the preferred binding sequence at the active site of the molecule. Broadly speaking, group-1 caspases bind YVAD derivatives and group-2 caspases bind DEVD. Aldehyde conjugates of these molecules make reversible inhibitors, while conjugation of the peptides to chloromethyl, fluoromethyl, or acyloxymethyl moieties give rise to irreversible inhibitors. These molecules form a covalent bond with the sulphur of the active cysteine within the large subunit. Further modifications provide fluorescent derivatives that make substrate molecules useful for assaying activity of different members of the caspase family.

# Caspases

CURRENT NAME	Caspase 1 ( <a href="#">C 5482</a> )	Caspase 2 ( <a href="#">C 2854</a> )	Caspase 3 ( <a href="#">C 1224</a> )	Caspase 4 ( <a href="#">C 6357</a> )	Caspase 5 ( <a href="#">C 6482</a> )	Caspase 6 ( <a href="#">C 4977</a> )
PREVIOUS NAME	ICE	ICH-IL Nedd-2 (mouse)	CPP32 YAMA/Apopain prICE (mouse)	TX, ICH-2 ICE-rel II	ICE-rel III, TY	Mch 2
PHYLOGENETIC FAMILY	1	2	3	1	1	3
SUBSTRATES	Pro-IL-1 $\beta$ Interferon- $\gamma$ inducing factor <sup>a</sup> Pro-Caspase 1 D4-GD1	Not PARP	PARP DNApk PKC- $\delta$ ( <a href="#">P 8538</a> ) Retinoblastoma SREBP 1,2 GAS 2 Huntingtin Fodrin (Spectrin) ICAD	Pro-Caspase 1 Pro-Caspase 4	Not Pro-IL-1 $\beta$ Pro-Caspase 5	PARP Lamins A, C
GROUP (SUBSTRATE)	1	II	II	I	I	III
PREFERRED SUBSTRATES	WEHD ( <a href="#">A 0216, A 6845</a> )	DEHD	DEVD ( <a href="#">A 1086, A 0466, A 2559</a> )	WEHD ( <a href="#">A 0216, A 6845</a> ) LEHD ( <a href="#">A 5970, A 5845</a> )	WEHD ( <a href="#">A 0216, A 6845</a> ) LEHD ( <a href="#">A 5970, A 5845</a> )	VEHD
INHIBITORS	Ac-YVAD-CHO ( <a href="#">A 3707</a> ) CrmA (nM) p35 Not IAP	Ac-DEVD-CHO ( <a href="#">A 0835</a> ) X-IAP (nM) p35	Ac-DEVD-CHO ( <a href="#">A 0835</a> ) X-IAP p35 CrmA Pro-caspase 3 ( <a href="#">P 1488</a> )	Ac-YVAD-CHO ( <a href="#">A 3707</a> ) CrmA (nM) p35	Ac-YVAD-CHO ( <a href="#">A 3707</a> ) CrmA	CrmA (nM) Not X-IAP
PRODOMAIN	Long	Long	Short	Long	Long	Short
BINDING MOTIFS	CARD	CARD	—	CARD	CARD	—
GRANZYME B ( <a href="#">G 9278</a> )	X	Activated	Activated	X	X	Activated

## ABBREVIATIONS

**APAF-1:** Apoptosis protease activating factor

**CARD:** Caspase recruitment domain

**CrmA:** Cytokine response modifier A

**ERICE:** Evolutionary related ICE

**FLICE:** Fas-associated death domain-like IL-1 $\beta$ -converting enzyme

**IAP:** Inhibitors of apoptosis

**ICAD:** Inhibitor of caspase-activated DNAase

**ICE:** Interleukin-1 $\beta$  converting enzyme

**MICE:** Mini ICE

**PARP:** Poly(ADP-ribose)polymerase-1

**PKC:** Protein kinase C

**SREBP:** Sterol regulatory element-binding protein

## FOOTNOTES

<sup>a</sup> Also known as interleukin-18.

## Caspases

CURRENT NAME <sup>a</sup>	Caspase 7 ( <a href="#">C 2979</a> )	Caspase 8 ( <a href="#">C 1099</a> )	Caspase 9 ( <a href="#">C 8726</a> )	Caspase 10 ( <a href="#">C 6607</a> )	Caspase 13	Caspase 14
PREVIOUS NAME	Mch 3 Ice-Lap3 CMH-1	MACH-H FLICE Mch 5	Ice-Lap6 Mch 6	Mch 4	ERICE	MICE
PHYLOGENETIC FAMILY	3	3	3	3	1	1
SUBSTRATES	SREBP 1,2 PARP HnRNP	Pro-Caspase 3 ( <a href="#">P 1488</a> ) Pro-Caspase 7	Pro-Caspase 3 ( <a href="#">P 1488</a> ) Pro-Caspase 7	?	?	—
GROUP	II	III	III	III	I	I
PREFERRED SUBSTRATES	DEVD ( <a href="#">A 1086</a> , <a href="#">A 0466</a> , <a href="#">A 2559</a> )	LETD ( <a href="#">A 6095</a> )	LEHD ( <a href="#">A 5845</a> , <a href="#">A 5970</a> )	LEHD ( <a href="#">A 5845</a> , <a href="#">A 5970</a> )	—	DEVD ( <a href="#">A 1086</a> , <a href="#">A 0466</a> , <a href="#">A 2559</a> )
INHIBITORS	Ac-DEVD-CHO ( <a href="#">A 0835</a> ) X-IAP	CrmA (nM), Not IAP	X-IAP p35?	CrmA	p35 CrmA	IAP, p35 Not CrmA
PRODOMAIN	Short	Long	Long	Long	Long	Short
BINDING MOTIFS	—	DED	CARD	DED	—	—
GRANZYME B ( <a href="#">G 9278</a> )	Activated	Activated	Activated	Activated	X	?

### FOOTNOTES

<sup>a</sup> mCaspase 11 and mCaspase 12 are long domain, group 1 caspases which have not been included since they are of murine origin.