Caspases: the central executioners of apoptosis

Procaspase 7
Multiple apoptosis pathways activate caspases
Discovery of Caspases

Cysteine-dependent aspartate specific protease

First implicated in apoptosis with the discovery that CED-3, which is a product of a gene required for cell death in *C. elegans*

CED-3 is similar to mammalian interleukin-1β-converting enzyme (*ICE-1* or caspase 1)

Represent a family of **cysteine proteases** that cleave after an Asp residue in their substrates, hence the name caspase

Caspases are all expressed as proenzymes (zymogens) which contain at least three domain, and activation of caspases involves proteolytic processing between domains.
The phylogenetic relationship correlates with their function

![Phylogenetic tree example](image-url)
Comparisons of caspases

DED: death effector domain

CARD: caspase activation and recruitment domain
Classification of caspases

Initiator caspases: caspase -2, -8, -9, -10

containing a long N-terminal pro-domain (near 90 amino acids)

effector caspases: caspase -3, -6, -7

containing a short N-terminal pro-domain (near 20-30 amino acids)
Introduction to caspases activation

**Classified to initiator caspases and effector caspases**

The initiator caspases are thought to be activated by auto-activation.

The effector caspases are activated by initiator caspases.

The functional unit of caspases is a homodimer, with each monomer consisting of a large subunit (20 kDa) and a small subunit (10 kDa).

Activation of initiator caspases is tightly regulated and often requires the assembly of a multicomponent complex under apoptotic conditions.
Proteolytic cleavage mediates activation of caspases

Science 1998, 281:1312
Activation of caspases

Long-prodomain caspases containing protein-protein interaction module, which allows it to bind to and associate with its upstream regulators

The first activation cleavage between the large and small subunits

Four surface loop structures form the catalytic groove

Removal of the pro-domain is unnecessary for its catalytic activity
The prodomains of caspases

**DED**: Caspase 8 and 10 contain a death-effector domain (DED)

The DED domains are required for association with death receptor and subsequent activation of the caspases

**CARD**: Several caspases contain a caspase activation and recruitment domain (CARD)

The CARD domains are found in caspases involved in both inflammation and apoptosis, so its role can be varied from caspase to caspase
The DED and CARD domains share little sequence identity, but fold into very similar three-dimensional structures.

The structures consist of six antiparallel $\alpha$ helices arranged in a Greek key configuration, which is also found in the death domain (DD), a third protein interaction module present in several upstream regulators of apoptosis, such as CD95 and the adapter FADD.
The structure of caspases

Homodimer, each monomer consists of a large subunit (p20) and a small subunit (p10)

1. Six antiparallel β strands from each catalytic subunit form interface for homodimerization which is mediated by hydrophobic interaction
2. Four protruding loops L1, L2, L3, and L4, forming a substrate binding groove, shape the active site
3. Loop L1 constitutes one side of the groove whereas L4 represents the other side
4. Loop L2 harbors the catalytic residue Cys
5. Loop L3 serves as the base of the active site
6. Loop L2' stabilizes the L2 and L4 loops

Mechanisms of activation of caspases
Activation of caspases 8

Caspase 8---Initiator caspases

Activation mechanism:
The clustering of caspase 8 zymogens possessing intrinsic enzymatic activity forces processing in trans manner

Evidence:

*In vivo*, activation of caspase 8, whose assembly is forced by ligation of death receptors, is mediated by specific adaptor proteins

*In vitro*, caspase 8 processes itself rapidly upon heterologous expression in *E. coli*, suggesting a significant intrinsic proteolytic activity

Nature 2000, 407:770
Activation of caspases 9

Mechanism:

**Activation of caspase 9 is probably mediated by conformation change**

The activation of caspase 9 occurs upon release of cytochrome c from the mitochondria.

Formation of an ~1.4 MDa complex, named apoptosome, by cytochrome c, Apaf-1 and procaspase 9 in the presence of dATP or ATP facilitates activation of caspase 9.

Caspase 9 does not have its N-peptide prodomain removed once activated.

Cleavage in the inter-domain linker is neither necessary nor sufficient for activation of caspase 9.
Activation of effector caspases

Effector caspases---caspase 3, 6, 7

They cannot be activated efficiently at physiological conditions via a homo-activation pathway, since they have no means of being recruited and activated like the initiator caspases.

The effector caspases rely on the action of the initiator caspases for their activation in vivo.

Effector caspase zymogens can be activated by proteases without specificity for Asp, at least in vitro; these enzymes include serine proteases Carlsberg, Cathepsin G, Granzyme B.
Why the similarity among caspases does not make effector caspases contain intrinsic catalytic activity like initiator caspase?
Activation of caspase 7

Studies on crystal structure of active caspase 7 indicates that:

Loop L3 is loosened above the base
L4 is located farther away from L3, thus flattening the active site pocket

L2, which contains the catalytic residue Cys186, is rotated 90°, making this residue inaccessible to solvent
L2’ loop is flipped 180°, still existing in a closed conformation

Ability of moving freely of L2’ determines the intrinsic activity; it is present in caspase 9 but not in caspase 7
Substrates of Caspases

Recognizing at least four contiguous amino acids, named P4-P3-P2-P1

P4-P3-P2-P1 (X-Glu-X-Asp)

Cleaving after P1, usually Asp; but some can be Glu
P3 is preferred to be an Glu

The known cellular substrates of caspases:
Other caspases
Structural components, such as actin and nuclear lamin
Regulatory components, such as DNA-dependent protein kinase, inhibitor of caspase-activated DNase (see table)
## Selective peptide substrates of caspases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Preferred peptide substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspase 1</td>
<td>YEVD</td>
</tr>
<tr>
<td>Caspase 2</td>
<td>VDVAD</td>
</tr>
<tr>
<td>Caspase 3</td>
<td>DMQD</td>
</tr>
<tr>
<td>Caspase 4</td>
<td>LEVD</td>
</tr>
<tr>
<td>Caspase 5</td>
<td></td>
</tr>
<tr>
<td>Caspase 6</td>
<td>VEID</td>
</tr>
<tr>
<td>Caspase 7</td>
<td>DEVD</td>
</tr>
<tr>
<td>Caspase 8</td>
<td>IETD</td>
</tr>
<tr>
<td>Caspase 9</td>
<td>LEHD</td>
</tr>
<tr>
<td>Caspase 10</td>
<td>Iead</td>
</tr>
</tbody>
</table>
Cellular substrates of caspases

<table>
<thead>
<tr>
<th>Polypeptides</th>
<th>Cleavage site</th>
<th>Responsible caspase</th>
<th>Proposed effect of cleavage</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Abundant cytoplasmic proteins</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gelsolin</td>
<td>DQTD/G</td>
<td>3</td>
<td>Calcium-insensitive actin cleavage</td>
<td>205, 206</td>
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<tr>
<td>Gas-2</td>
<td>SRVD/G</td>
<td>?</td>
<td>Cytoskeleton rearrangement</td>
<td>207</td>
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<tr>
<td>Fodrin (Alpha II-spectrin)</td>
<td>DETD/S</td>
<td>3</td>
<td>Plasma membrane blebbing</td>
<td>208, 210, 211, 211a, 366</td>
</tr>
<tr>
<td></td>
<td>DSLD/S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta II-spectrin</td>
<td>DEVD/S</td>
<td>3</td>
<td>Unknown</td>
<td>211a</td>
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<tr>
<td>Beta-Catenin</td>
<td>?</td>
<td>3</td>
<td>↓α-Catenin binding and cell-cell contact</td>
<td>212, 213</td>
</tr>
<tr>
<td>Cytokeratin 18</td>
<td>VEVD/A</td>
<td>3, 6, 7</td>
<td>?</td>
<td>216, 217</td>
</tr>
<tr>
<td>Abundant nuclear proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamin A</td>
<td>VEID/N</td>
<td>6</td>
<td>Nuclear lamina disassembly</td>
<td>12, 44, 220, 223</td>
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<tr>
<td>Lamin B1</td>
<td>VEVD/S</td>
<td>6, ?3</td>
<td>Nuclear lamina disassembly</td>
<td>10–12, 104, 221–223</td>
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<tr>
<td>NuMA</td>
<td>?</td>
<td>3, 6</td>
<td>Nuclear shape changes</td>
<td>131, 226, 228–231</td>
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<tr>
<td>HnRNP proteins</td>
<td>?</td>
<td>3</td>
<td>↓RNA processing</td>
<td>222</td>
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<tr>
<td>C1 and C2</td>
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<tr>
<td>70-kDa protein of U1 snRNP</td>
<td>DGPD/G</td>
<td>3</td>
<td>↓RNA processing</td>
<td>226, 223, 234, 242</td>
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<tr>
<td>mdr2</td>
<td>DVPD/C</td>
<td>3, 6, 7</td>
<td>Unknown, still binds p53</td>
<td>236, 237</td>
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<tr>
<td>Proteins involved in DNA metabolism and repair</td>
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<tr>
<td>PARP</td>
<td>DEVD/G</td>
<td>3, 7, 9</td>
<td>↓Synthesis of poly(ADP-ribose)</td>
<td>10, 12, 62, 132, 197, 226, 242, 367–369</td>
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<tr>
<td>DNA-PKes</td>
<td>DEVD/N</td>
<td>3</td>
<td>↓Activity in some studies</td>
<td>238–243</td>
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</tbody>
</table>
Cellular substrates of caspases

<table>
<thead>
<tr>
<th>Protein</th>
<th>Substrates</th>
<th>Function</th>
<th>IC</th>
</tr>
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<tbody>
<tr>
<td>p27kip1</td>
<td>DPSD/S</td>
<td>-p27 in cyclin E-cdk complexes</td>
<td>288</td>
</tr>
<tr>
<td>Rb (retinoblastoma) protein</td>
<td>DEAD/C</td>
<td>Unopposed E2F-1 action</td>
<td>209, 210</td>
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<tr>
<td>CDC27</td>
<td>?</td>
<td>Ubiquitin ligase, stabilization of cyclins A and B</td>
<td>272</td>
</tr>
</tbody>
</table>

Proteins Involved in Human Genetic Diseases

<table>
<thead>
<tr>
<th>Protein</th>
<th>Substrates</th>
<th>Function</th>
<th>IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huntington</td>
<td>DSVD/L</td>
<td>Possible nonphysiological cleavage (cf. 372)</td>
<td>293, 294</td>
</tr>
<tr>
<td>Dentatorondal pallidalsis atrophy protein</td>
<td>DK1/D/G</td>
<td>No known effect</td>
<td>294, 295</td>
</tr>
<tr>
<td>Presenilin-1</td>
<td>ARQD/S</td>
<td>Unknown</td>
<td>296–298</td>
</tr>
<tr>
<td>Presenilin-2</td>
<td>DSYD/S</td>
<td>Generates antiapoptotic fragment</td>
<td>298, 297, 299</td>
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</table>

Apoptotic regulatory proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Substrates</th>
<th>Function</th>
<th>IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2</td>
<td>DAGD/V</td>
<td>Generates proapoptotic fragment</td>
<td>301–304</td>
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<tr>
<td>Bcl-xT</td>
<td>HLAD/S</td>
<td>Generates proapoptotic fragment</td>
<td>305</td>
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<tr>
<td>FLIP L</td>
<td>LEVD/G</td>
<td>Unknown</td>
<td>144, 147</td>
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<tr>
<td>Bid</td>
<td>LOTD/G</td>
<td>Generates proapoptotic fragment</td>
<td>306, 307</td>
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<tr>
<td>Bax</td>
<td>FIQD/R</td>
<td>Unknown</td>
<td>303</td>
</tr>
<tr>
<td>ICAD</td>
<td>DEPD/S</td>
<td>Liberates active CAD endonuclease</td>
<td>308, 309, 312, 345</td>
</tr>
</tbody>
</table>

See also HuRNP particle proteins C1 and C2 as well as the U1 snRNP particle 70 kDa polypeptide in other parts of this table.

See also HuRNP particle proteins C1 and C2 as well as the U1 snRNP particle 70 kDa polypeptide in other parts of this table.

In addition, all of the proapoptosis are caspase substrates as indicated in the text and Table 1.
Regulation of caspases

I. Transcriptional regulation
   Under apoptotic condition

II. Posttranslational modification
   Phosphorylation inactivation of caspase 9 by Akt
   Ubiquitination-mediated proteasome degradation

III. Inhibition by IAP (inhibitor of apoptosis)
IAPs : share common baculoviral IAP repeat (BIR) domain

IAP (Inhibitor of apoptosis)

IAP targets and inhibits caspases by **baculoviral IAP repeat** (BIR) domains

Original identified in the baculovirus (p35) based on their ability to suppress apoptosis in infected cells

BIR contains ~80 amino acids folded around a zinc atom

XIAP, c-IAP1, and c-IAP2 contain three BIR domains
The third BIR domain (BIR3) inhibits caspase-9
The linker region between BIR1 and BIR2 targets caspase-3 and -7

These IAPs target the caspases, including caspase -9, -3, -7; but not other caspases

RING-containing E3 ligase activity and IAP function

Studies in fly (D. melanogaster)

The key function of a RING finger is to bind an E2 enzyme (UBC)

Heterozygous mutation in RING of DIAP1 are more sensitive to apoptosis induced by overexpression of Reaper

Homozygous mutation in RING of DIAP1 results in embryonic lethal

RHG proteins and caspases have been shown to be substrates of the E3 ligase activity of IAP in vitro

RHGs targets/targetted by RING containing E3 ligase activity of IAP

IAP promotes ubiquitylation of RHG proteins: Reaper, Hid, Grim in vitro

Autoubiquitylation of IAP promoted by RHG proteins in vitro

Model:
IAP binding to RHGs activates the autoubiquitylation activity of the IAP, and both the IAP and bound RHGs are degraded by the proteasome.
Mechanisms of IAP-mediated inhibition of caspases (I)

Inhibition of caspase -3 and -7

Linker segment of XIAP occupies the active site of caspases, blocking the substrate entry

Asp148, Val146, and Gly144 bind to S4 pocket, S2 pocket, and S1 pocket of caspases, respectively.

Linker fragment alone cannot inhibit caspases; it works with surrounding domains, suggesting a productive conformation by surrounding domains.

Mechanisms of IAP-mediated inhibition of caspases (II)

Inhibition of caspase 9 by XIAP

Both BIR2 and BIR3 of XIAP can inhibit caspase-9

BIR3 displays tighter binding affinity and higher potency

mutation in Trp310 and Glu314 of BIR3 completely abrogates XIAP-mediated inhibition
Ablation of eye structures due to overexpression of RHG proteins in eyes of Drosophila

However, deletions of RHG genes completely block apoptosis during embryogenesis and cause embryonic lethality.

p35 is a baculovirus gene which blocks apoptosis to help viral replication in insect cells.
Covalent inhibition of caspases by p35

Baculoviral p35 protein is a pan-caspase inhibitor

the catalytic residue Cys360 of caspase-8 is covalently linked to the Asp87 of p35 through a thioester bond
The RHG family in Fly apoptosis

Death-promoting RHG Family proteins

The RHG members in mammalian apoptotic pathway

Smac/DIABLO    Omi/HtrA2
**Smac/DIABLO: inhibitor of IAP**

Apoptosis-induced release of Smac/DIABLO from mitochondria to the cytosol

Smac/DIABLO interacts with multiple IAPs and counteracts IAP-mediated inhibition of caspases

Functions as an elongated dimer, and wild-type Smac binds to both the BIR2 and the BIR3 domains of XIAP but not the BIR1 domain

N-terminal residues of Smac (after cleavage of mitochondrial targeting peptide) play a pivotal role in Smac function

The Smac N-terminal tetrapeptide (Ala-Val-Pro-Ile) interacting with an acidic surface groove on BIR3 mediates the inhibition on IAP

The similarity between Smac/DIABLO and caspase-9

The N-terminal tetrapeptide (AVPI) of Smac is similar to the RHG motif of the RHG proteins in Drosophila.

An internal tetrapeptide motif (ATPF), named IBM, in caspase-9 mediates binding between caspase-9 and IAP.

IBM: The conserved IAP-binding motif in caspase-9 and Smac mediates opposing effects on caspase activity.

The similarity between AVPI (Smac) and ATPF (Caspase 9) in binding to XIAP

**Figure 9**

- **Mammals:**
  - Smac/DIABLO
  - hCasp-9
  - mCasp-9
  - zCasp-9
  - hTrxA2/omi
  - mTrxA2/omi

  - AVPLACKS
  - ATRPPSEQL
  - AVPVEGFP
  - ATVPFGSE
  - AVPSPPPA
  - AVPAPPPT

- **Drosophila:**
  - Reaper
  - Grim
  - Hid
  - Sickle
  - Jafrac2

  - AVAPYLDP
  - ATAYPLDP
  - AVFYLPX
  - ATPFIERE
  - AKPEDNES

- AVPI (Smac) bound to BIR3 of XIAP
- ATPF (Caspase-9) bound to BIR3 of XIAP
The overview of caspase activation and inhibition regulated by Smac and IAP
References


