Cell Death and Differentiation (2002) 9, 358–361

www.nature.com/cdd

News and Commentary

Alice in caspase land. A phylogenetic analysis of caspases from worm to man

M Lamkanfi[1], W Declercq[1], M Kalai[1], X Saelens[1]
P Vandenabeele*,[1]

[1] Molecular Signalling and Cell Death Unit, Department of Molecular Biology, Flanders Interuniversity Institute for Biotechnology and University of Gent, Ledeganckstraat 35, B-9000 Gent, Belgium
* Corresponding author: E-mail: Peter.Vandenabeele@dmb.rug.ac.be
DOI: 10.1038/sj/cdd/4400989

Caspases belong to a conserved family of cysteinyl aspartate proteinases that are involved in metazoan programmed cell death and inflammation. To date 11 human, 10 murine, four avian, four fish, eight amphibian, seven insect and three nematode caspases have been identified. An evolutionary related family of cysteine proteases, viz. metacaspases (found in plants, fungi and protozoa) and paracaspases (found in metazoans and *Dictyostelium*), also contain a conserved Cys-His catalytic diad.[1] Caspases consist of a prodomain of variable length, followed by a p20 and a p10 unit that contain the residues essential for substrate recognition and catalysis (Figure 1A). Caspases are activated by proteolysis, separating the p20 and p10 subunits, allowing their reassembly as an active heterotetramer. The phylogenetic tree presented in Figure 1B is based on the amino acid sequence containing the p20 and p10 units, referred to as p30 caspase. The prodomain was excluded from the phylogenetic analysis because it contains recruitment motifs unrelated to the catalytic properties of caspases and because these motifs also occur in other proteins. Moreover, since downstream caspases lack such a prodomain, a homology comparison including prodomain sequences is somewhat biased. For similar reasons the meta- and paracaspases were compared on the basis of their p20-like subunit (Figure 1C).

A phylogenetic analysis of p30 caspases in *Caenorhabditis elegans, Drosophila melanogaster, Xenopus laevis, Danio rerio, Gallus gallus, Mus musculus* and *Homo sapiens* showed that they diverge in three main clusters (Figure 1B). Interestingly, all *C. elegans* caspases gather in cluster I, which contains also the caspase-1-like caspases as well as caspase-2 and -14. All apoptosis-related executioner caspases belong to cluster II and have a short prodomain, with the exception of *Drosophila* STRICA. The main initiator apoptotic caspases gather in cluster III.

The first cluster contains two branches: one comprising *C. elegans* caspases and a second consisting solely of caspase-1, -2, -4, -5, -11, -12 and -14, so far only found in vertebrates. The *C. elegans* CED-3 is the godfather of the caspase family. It was the first gene product identified to be essential for programmed cell death.[2] CSP-1 and CSP-2 have a long prodomain without obvious functional motifs. Little is known about their possible involvement in proteolytic cascades. In the nematode the apparent lack of short prodomain caspases is bypassed by alternative splicing of CSP-1 and -2. However, protease activity has only been reported for CED-3 and CSP-1.[3] The caspases in the second branch do not have orthologues in the fly and the nematode, as deduced from their genome sequences. Thus this group of caspases may have evolved together with a complex hematopoietic system implicated in inflammatory and immune responses. The prototype of this group is caspase-1, which is mainly implicated in the processing of inflammatory cytokines such as proIL-1β and IL-18.[4,5] Therefore this group is often referred to as inflammatory caspases, a name that is supported by the inability of LPS to induce endotoxemia in caspase-1- and caspase-11- deficient mice.[4–6] In this respect the role of xcaspase-1 is not yet clear, as the *Xenopus* proIL-1β orthologue seems to lack a clear caspase-sensitive cleavage site at the appropriate position.[7] Caspase-1 can also be activated in response to binding of bacterial lipoproteins to Toll-like receptor 2, suggesting a link between the Toll receptor system and the activation of inflammatory caspases.[8] Similar to procaspase-9 recruitment by Apaf-1, a novel Apaf-1-like factor, viz. Ipaf, has been described which recruits and activates procaspase-1 in inflammatory and proapoptotic conditions.[9] The precise human orthologues of murine caspase-11 and caspase-12 are not yet known. It can be argued that human caspase-4 and -5 are duplicated counterparts of murine caspase-11. When comparing human procaspases, procaspase-4 and -5 have an amino acid sequence identity of 77%, the next highest identity score being 55% (between procaspase-1 and -4). Procaspase-4 and -5 amino acid sequences are 59 and 54% identical to procaspase-11, respectively. They are only 48 and 45% identical to procaspase-12, respectively. Furthermore, caspase-4 and -11 mRNA have similar tissue distribution patterns.[10] Both caspase-5 and -11 are LPS- or IFN-γ-inducible.[11] Caspase-12 has been reported to be an ER stress-sensing protease.[12] That thirteen is an unlucky number is confirmed again: the name human caspase-13 has erroneously been attributed to a caspase of bovine origin. Most probably this caspase is a bovine orthologue of caspase-4.[13] Surprisingly, chicken caspase-1 lacks a large prodomain.[14] Caspase-2 has deviated from the main branch leading to the caspase-1-likes and is implicated in neuronal cell death, suggesting an apoptotic initiator function for this caspase.[15] Nevertheless, like caspase-1, caspase-2 can mediate apoptosis of macrophages infected with *Salmonella*.[16] The CARD domain of procaspase-2 is most related to that of procaspase-9. This resemblance in the prodomain is

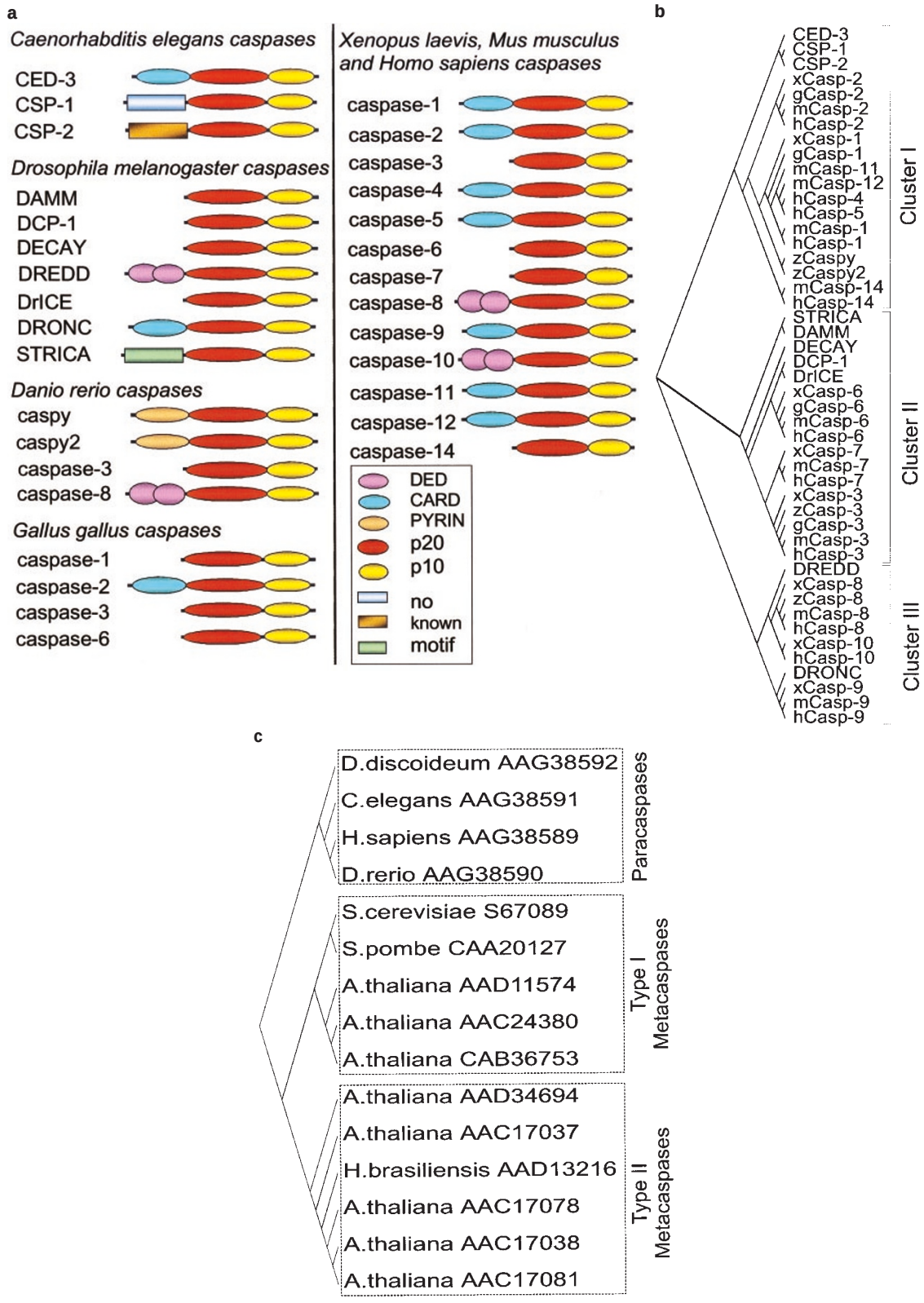

Figure 1 (**A**) Domain architecture of the caspases in mammals, chicken, *Xenopus*, zebrafish, *Drosophila* and *C. elegans*. (**B**) Phylogenetic relationship of the caspases based on their p20 and p10 domains. The inflammatory caspases (cluster I), the apoptotic executioner caspases (cluster II) and the apoptotic initiator caspases (cluster III) evolved as separate groups. The sequences were aligned using the CLUSTAL X algorithm (gap weight=10.00; gap length weight=0.20). *Danio rerio* (z), *Xenopus laevis* (x), *Gallus gallus* (g), *Mus musculus* (m), *Homo sapiens* (h). (**C**) Phylogenetic relationship of the para- and metacaspases. The paracaspases, the type I and type II metacaspases segregate in three clusters. The proteins are indicated by their NCBI accession numbers. The sequences were aligned using the CLUSTAL X algorithm (gap weight=10.00; gap length weight=0.20) using the p20-like subunits of the para- and metacaspases

reflected in the observation that both procaspase-2 and procaspase-9 specifically interact with the same proapoptotic caspase adaptor protein PACAP, a cytochrome *c* and ATP-binding protein that promotes the proteolytic activation of these caspases.[17] In addition, the CARD domain of caspase-2 allows its recruitment in the TNF-R1 complex through binding to the CARD and DD-domain-containing adaptor RAIDD.[18] However, a precise function in this respect has not yet been found. Zebrafish caspy and caspy2 are the orthologues of mammalian caspase-1 and -2, respectively, though their prodomains contain a PYRIN motif instead of a CARD.[19] Caspase-14 is a short prodomain caspase, mainly expressed and processed in differentiating keratinocytes of the skin.[20] Caspase-14 does not participate directly in the apoptotic cell death cascades, but has been proposed to contribute to the specialized form of programmed cell death in the skin. However, the molecular mechanism of caspase-14 proteolysis is not yet clear.

Cluster II contains the executioner caspases of all species examined (Figure 1B). The apoptotic proteolytic cascades and substrates have been extensively described for mammalian executioner caspases.[21,22] Caspase-6 takes a somewhat separate position in the group of executioner caspases as it deviated from the branch leading to the closely related caspase-3 and -7. *Xenopus* contains both caspase-3 and -7, while caspase-7 has not yet been reported in fish and chicken. However, caspase-3 and -7 cannot be considered as completely redundant caspases in view of gene-targeting experiments that revealed profound phenotypic differences.[23] Although caspase-3 and -7 share many substrates, they also exhibit distinct substrate specificities, e.g. endothelial macrophage-activating factor II (EMAP-II) and certain procaspases.[21,24] In *Drosophila melanogaster* the short prodomain caspases DECAY, DCP1 and DRICE are considered as downstream effector caspases based on structural and enzymatic properties.[25,26] DAMM and STRICA have a related p30 domain and have highly diverged from the other *Drosophila* caspases.[26] STRICA has an atypically large prodomain consisting of many Ser and Thr residues, suggesting that phosphorylation regulates the activity of this caspase.[27] DAMM is a short prodomain caspase (Figure 1A).

Cluster III harbours two main branches of initiator caspases, one leading to caspase-8 homologues (DREDD, caspase-8, caspase-10) and another leading to caspase-9 homologues (DRONC and caspase-9). Both classes of procaspases have a prodomain with recruitment motifs, two DEDs in the caspase-8 homologues and a CARD in the caspase-9 homologues. It is remarkable that the catalytic p30 segregate in accordance with their respective recruitment motifs in the prodomain. Since only the caspase-8-like branch exhibits a DED motif, whereas CARD motifs are found both in cluster I and III, it can be argued that the DED motif has evolved later, reflecting a demand for specific recruitment in receptor complexes. This may correlate with the origin of a dichotomy between extrinsic and intrinsic apoptotic cell death pathways. The former is initiated by DED-containing caspases, whereas the latter depends on CARD-containing caspases for the formation of an apoptosome complex.

The first branch of cluster III includes the upstream initiator caspase-8 in *M. musculus*, caspase-8 and caspase-10 in *X. laevis* and *H. sapiens*, and DREDD in *D. melanogaster*. Since both caspase-8 and caspase-10 have been identified in *X. laevis* and man, the apparent absence of caspase-10 in mice is remarkable. Caspase-8 and caspase-10 contain a prodomain characterized by the presence of two DED motifs. The latter allow recruitment of these caspases in death receptor complexes following ligand binding, leading to proximity-induced activation of the proenzymes. In the fly, DREDD shares many features with procaspase-8, such as the presence of two DED motifs in the prodomain and the interaction with a FADD-like caspase adaptor protein, called dFADD.[28] dFADD also contains a C-terminal DD motif; however, in the fly no DD receptors have been reported so far.[28] DREDD also seems to play a role in the innate immunity signalling pathway of the Toll receptor by proteolytically processing Relish, an NF-κB-like transcription factor.[29] In mammalians, a link between the Toll-like receptor 2 and caspase-8 has been demonstrated through the MyD88-dependent recruitment of FADD.[8] Moreover, by analogy with DREDD, caspase-8 can promote activation of NF-κB.[30]

The second branch of cluster III contains procaspase-9 and its functional equivalent DRONC. In addition to the resemblance of the p30 they share a similar CARD motif in the prodomain and interact with their corresponding Apaf-1 orthologues, called DARK/dAPAF-1/HAC-1 in *D. melanogaster*.[31] Moreover, both DARK and Apaf-1 activate procaspase-9 and DRONC in a cytochrome *c* and dATP-dependent way.[31] These data suggest a similar assembly of the apoptosome complex both in the fly and in mammals, defining the initiation of the intrinsic apoptotic cell death pathway. The apoptosome complex formed in the nematode is peculiar since CED-4, the functional homologue of Apaf-1, does not contain the WD-40 repeats required for the binding of cytochrome *c*, and seems to activate the CARD domain containing CED-3 in a cytochrome *c* independent way.[32]

Human caspases have been classified before in three groups based on screening of a combinatorial tetrapeptide substrate library.[33] Group I caspases (caspase-1, -4, -5) prefer bulky hydrophobic residues at the P4 position of the substrate. Group II caspases (caspase-2, -3, -7, CED-3) have a strict requirement for an Asp in the P_4 position and mostly mediate the cleavage of cellular proteins during apoptosis. Group III caspases (caspase-6, -8, -9 and-10) prefer a branched chain aliphatic amino acid residue at the P4 position. This classification meets the phylogenetic ordering of the p30 caspases in three clusters (Figure 1B). Only caspase-2 and -6 have a tetrapeptide substrate specificity different from that of the cluster they belong to.

Two distinct families of caspase-like proteins, viz. paracaspases (found in metazoans and *Dictyostelium*) and metacaspases (found in plants, fungi and protozoa), have recently been identified.[1] These families of cysteinyl proteases only share with the caspases the conserved Cys-His catalytic diad within a p20-like subunit, but lack a p10 subunit. Therefore, the phylogenetic relationship of the different para- and metacaspases in Figure 1C is based on

the p20-like domain. Metazoan paracaspase prodomains contain a DD motif and immunoglobulin-like domains. It is clear that the metacaspases segregate in two groups, designated type I and type II metacaspases. The type I metacaspases from fungi and plants have large prodomains with a proline-rich repeat motif. Many plant type I metacaspases have in addition a zinc finger motif similar to that of the plant hypersensitive response/cell death protein Isd-1. Type II metacaspases (in plants) have no prodomain, but instead have a 200 amino acid C-terminal extension. The caspase, paracaspase and metacaspase families belong to a distinct superfamily within the thiol proteases. This superfamily also includes legumains, hemoglobinases and bacterial gingipain R, which has recently been shown to have a caspase fold, despite little sequence homology with caspases.[34,35] This suggests that bacterial cysteine proteases and eukaryotic caspases, paracaspases and metacaspases may have a common ancestor. Only one mammalian paracaspase has been identified so far (MLT/MALT1). It may play a central role in the oncogenesis of mucosa-associated lymphoid tissue lymphoma (MALT lymphoma) due to interaction with the NF-κB-activating Bc110 gene product leading to enhanced anti-apoptotic signalling.[1,36]

Caspases have already provided a lot of surprises. Even as close spectators of an exciting and fast developing field we often feel like Alice in Wonderland encountering many different appearances of caspase-related effects. What makes caspases so interesting? First, they can be recruited to complexes and organelles by virtue of motif-containing prodomains. Second, this recruitment allows proximity-induced activation. Third, they have a fairly specific proteolytic activity, allowing mediation of signalling events instead of mere protein degradation as occurs in proteasomes. Fourth, they operate in specific intracellular protease cascades, permitting strong and irreversible amplification of a signal. These features graft specific proteolysis onto a world of spatial and temporal organisation of cellular signalling. The same principle of combining the specificity of proteases and recruitment motifs may apply to the paracaspases and the type II metacaspases. In these protease families variants without prodomain also occur, viz. the type I metacaspases. The latter may function as executioners activated by initiator cysteine proteases.

In *C. elegans*, caspases seem to function mainly in programmed cell death. In higher organisms caspase genes diversified and acquired additional functions. In *Drosophila*, initiator caspase genes (cluster III) and short prodomain executioner caspase genes (cluster II) are already apparent. However, no cluster I inflammatory caspases are present in the fly, though DREDD seems to fulfil a role in innate immunity. In vertebrates a voluptuous cluster I developed with caspases implicated in inflammation and infection. In general, a remarkable co-evolution occurred between the p30 catalytic domain and the recruitment motif containing prodomain of the caspases.

Acknowledgements

This work was supported by the Interuniversitaire Attractiepolen IV/26, the Fonds voor Wetenschappelijk Onderzoek – Vlaanderen (grant 3G.0006.01), the Bijzonder Onderzoeksfonds, and an EC-RTD grant QLRT-1999-00739. M Lamkanfi is a fellow with the Vlaams Instituut voor de Bevordering van het Wetenschappelijk-technologisch Onderzoek in de Industrie. X Saelens is supported by the 'Biotech Fonds'.

1. Uren AG *et al.* (2000) Mol. Cell 6: 961 – 967
2. Ellis HM and Horvitz HR (1986) Cell 44: 817 – 829
3. Shaham S (1998) J. Biol. Chem. 273: 35109 – 35117
4. Li P *et al.* (1995) Cell 80: 401 – 411
5. Ghayur T *et al.* (1997) Nature 386: 619 – 623
6. Wang S *et al.* (1998) Cell 92: 501 – 509
7. Zou J *et al.* (2000) Immunogenetics 51: 332 – 338
8. Aliprantis AO *et al.* (2000) EMBO J. 19: 3325 – 3336
9. Poyet JL *et al.* (2001) J. Biol. Chem. 276: 28309 – 28313
10. Van de Craen M *et al.* (1997) FEBS Lett. 403: 61 – 69
11. Lin XY, Choi MS and Porter AG (2000) J. Biol. Chem. 275: 39920 – 39926
12. Nakagawa T *et al.* (2000) Nature 403: 98 – 103
13. Koenig U *et al.* (2001) Biochem. Biophys. Res. Commun. 285: 1150 – 1154
14. Johnson AL *et al.* (1998) Gene 219: 55 – 62
15. Troy CM *et al.* (2000) J. Neurosci. 20: 1386 – 1392
16. Jesenberger V *et al.* (2000) J. Exp. Med. 192: 1035 – 1046
17. Bonfoco E *et al.* (2001) J. Biol. Chem. 276: 29242 – 29250
18. Duan H and Dixit VM (1997) Nature 385: 86 – 89
19. Inohara N and Nunez G (2000) Cell Death Differ 7: 509 – 510
20. Lippens S *et al.* (2000) Cell Death Differ 7: 1218 – 1224
21. Van de Craen M *et al.* (1999) Cell Death Differ 6: 1117 – 1124
22. Nicholson DW (1999) Cell Death Differ 6: 1028 – 1042
23. Zheng TS *et al.* (1999) Cell Death Differ 6: 1043 – 1053
24. Behrensdorf HA *et al.* (2000) FEBS Lett. 466: 143 – 147
25. Adrain C and Martin SJ (2001) Cell Death Differ 8: 319 – 323
26. Kumar S and Doumanis J (2000) Cell Death Differ 7: 1039 – 1044
27. Doumanis J *et al.* (2001) Cell Death Differ 8: 387 – 394
28. Hu S and Yang X (2000) J. Biol. Chem. 275: 30761 – 30764
29. Leulier F *et al.* (2000) EMBO Rep. 1: 353 – 358
30. Hu WH *et al.* (2000) J. Biol. Chem. 275: 10838 – 10844
31. Kanuka H *et al.* (1999) Mol. Cell 4: 757 – 769
32. Chinnaiyan AM *et al.* (1997) Science 275: 1122 – 1126
33. Thornberry NA *et al.* (1997) J. Biol. Chem. 272: 17907 – 17911
34. Barrett AJ and Rawlings ND (2001) Biol. Chem. 382: 727 – 733
35. Eichinger A *et al.* (1999) EMBO J. 18: 5453 – 5462
36. Lucas PC *et al.* (2001) J. Biol. Chem. 276: 19012 – 19019