Classification and nomenclature of metacaspases and paracaspases: no more confusion with caspases

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Metacaspases and paracaspases are proteases that were first identified as containing a caspase-like structural fold (Uren et al., 2000) known as the caspase-hemoglobinase fold (CHF, Aravind and Koonin, 2002). Like caspases, meta- and paracaspases are multifunctional proteins regulating diverse biological phenomena, such as aging, immunity, proteostasis and programmed cell death. The broad phylogenetic distribution of meta- and paracaspases across all kingdoms of life and large variation of their biochemical and structural features complicate classification and annotation of the rapidly growing number of identified homologs. Establishment of an adequate classification and unified nomenclature of meta- and paracaspases is especially important to avoid frequent confusion of these proteases with caspases - a tenacious misnomer that unfortunately does not appear to decline with time. This letter represents a consensus opinion of researchers studying different aspects of caspases, meta- and paracaspases in various organisms, ranging from microbes to plants and animals.

Classification of meta- and paracaspases

The current classification of proteases provided by the MEROPS database clusters caspases, meta- and paracaspases to the same family, C14, within the CD clan (https://www.ebi.ac.uk/merops/). All members of C14 family are annotated to possess aspartate P1 cleavage specificity, and the family is further split into two subfamilies: C14A (caspases) and C14B (meta- and paracaspases).

Importantly, the MEROPS approach of grouping proteases into families or subfamilies is based on statistically significant similarities of the amino acid sequence within the peptidase domain or part thereof, without considering their biochemical properties (Rawlings et al., 2018). Being valuable for high-throughput protease classification, this approach, however, has substantial drawbacks if implemented without further adjustment. Indeed, in contradiction with the MEROPS description, none of the meta- or paracaspases characterized so far cleave after an aspartate residue. Instead, paracaspases are arginine-specific (Coornaert et al., 2008; Hachmann et al., 2012; Rebeaud et al., 2008), whereas metacaspases can cleave after either arginine or lysine (**Fig 1A;** Sundström et al., 2009; Vercammen et al., 2004). Such fundamental differences in the proteolytic specificity between caspases, meta- and paracaspases imply distinct repertoires of new proteoforms that they generate and point to the complex diversification and coevolution of their substrates and downstream pathways. One unfortunate consequence of the current classification is the misuse of

caspase-specific probes for studying meta- and paracaspases that is commonly found in the literature and leads to false conclusions.

Apart from substrate specificity, caspases, meta- and paracaspases feature other fundamental differences (**Fig. 1A**). For example, active metacaspases are monomers and their activation usually requires millimolar concentrations of calcium (Hander et al., 2019; McLuskey et al., 2012; Wong et al., 2012). In contrast, active caspases and paracaspases are calcium-independent dimers (Hachmann et al., 2012; Weismann et al., 2012; Yu et al., 2011). This indicates that upstream pathways regulating activation of caspases, meta- and paracaspases are likewise different.

In the past two decades we have learned about important differences between caspases, meta- and paracaspases. Thus, simple extrapolation of features typical for caspases to all other members of the C14 family is not justified anymore. Instead caspases, meta- and paracaspases should be separated into three corresponding groups within the family and each group should be properly annotated by providing its key biochemical and structural characteristics. We kindly request curators of MEROPS database to make corresponding changes.

Since structure and substrate specificity of prokaryotic caspase-like proteases named "orthocaspases" remain largely unknown (Klemenčič et al., 2015) we leave their classification and nomenclature open until their structural and biochemical properties have been clarified.

Unified nomenclature of meta- and paracaspases

The name "caspase" stands for "cysteine-dependent aspartate-specific protease". Thus, the names "metacaspase" and "paracaspase" imply the wrong substrate specificity for these proteases. However, since these names have been used for two decades we propose to keep them, provided that caspases, meta- and paracaspases are recognized as three separate groups within the C14 family.

Based on domain composition and arrangement, meta- and paracaspases are further sub-divided into three and two types, respectively (**Fig. 1A**). For the sake of consistency, we propose to maintain a common nomenclature for the different types of meta- and paracaspases using Latin

numerals (e.g. type I metacaspases). As for the conserved protein structures, they will be referred to as the p20-like region, the p10-like region, the linker region and the N-terminal pro-domain, matching the nomenclature of caspases (**Fig. 1A;** Alnemri et al., 1996). The p20, p10 and linker regions have been previously defined for the caspase group of the C14 family (Fuentes-Prior and Salvesen, 2004) and can be easily identified in meta- and paracaspase homologs based on a hidden Markov model (HMM) alignment with the C14 peptidase domain (PF00656) (**Fig. 1B**). Notably, although not always clearly stated in the literature, most known members of the C14 family contain the linker region. Furthermore, type II metacaspases are distinguished by a long linker between the p20 and p10 regions and an additional linker within the p10 region (**Fig. 1A**), which are frequently referred to as a single linker.

We suggest to consider the active form of meta- or paracaspases being a monomer if it is a cleaved or intact polypeptide chain derived from a single translational event, and a dimer if it comprises uncut or processed products of two translational events.

We propose to optimize nomenclature recommendations previously established for *Arabidopsis thaliana* metacaspases (Tsiatsiani et al., 2011) and vertebrate paracaspases (Hulpiau et al., 2016) in order (i) to have a unified nomenclature across meta- and paracaspase groups of the C14 family, (ii) to facilitate comparison of orthologs from different organisms, and (iii) to make it suitable for annotating homologs of species with partially sequenced genomes. Thus, we suggest using simple root symbols such as MCA for metacaspases and PCA for paracaspases. When naming individual family members, these root symbols will be preceded by the abbreviated Latin name of the species and followed by a hyphen, Latin number representing the type and then a small alpha character indicating in alphabetical order the number of the homolog of this type in a given genome (**Fig. 1C**). Proenzymes that require proteolytic processing for activation could be annotated with a prefix "pro-", e.g. pro-AtMCA-Ia for the metacaspase 1 of type I from *A. thaliana*. Spliceoforms should be indicated by a decimal number (e.g. AtMCA-Ia.1). Please note that these conventions do not consider the letter case, which should conform to gene and protein nomenclature established for a given model organism or taxonomic group.

Importantly, this nomenclature should be used synonymously for meta- and paracaspase homologs with well-established names, e.g. human MALT1/HsPCA-Ia or *A. thaliana* AtMC1/AtMCA-Ia. We encourage all researchers to adopt these recommendations. The new classification and unified nomenclature of meta- and paracaspases will facilitate a more comprehensive exchange of relevant findings within the scientific community and help to bridge already existing knowledge with newly discovered homologs, thus promoting mechanistic understanding of these ancient, evolutionarily conserved proteases.

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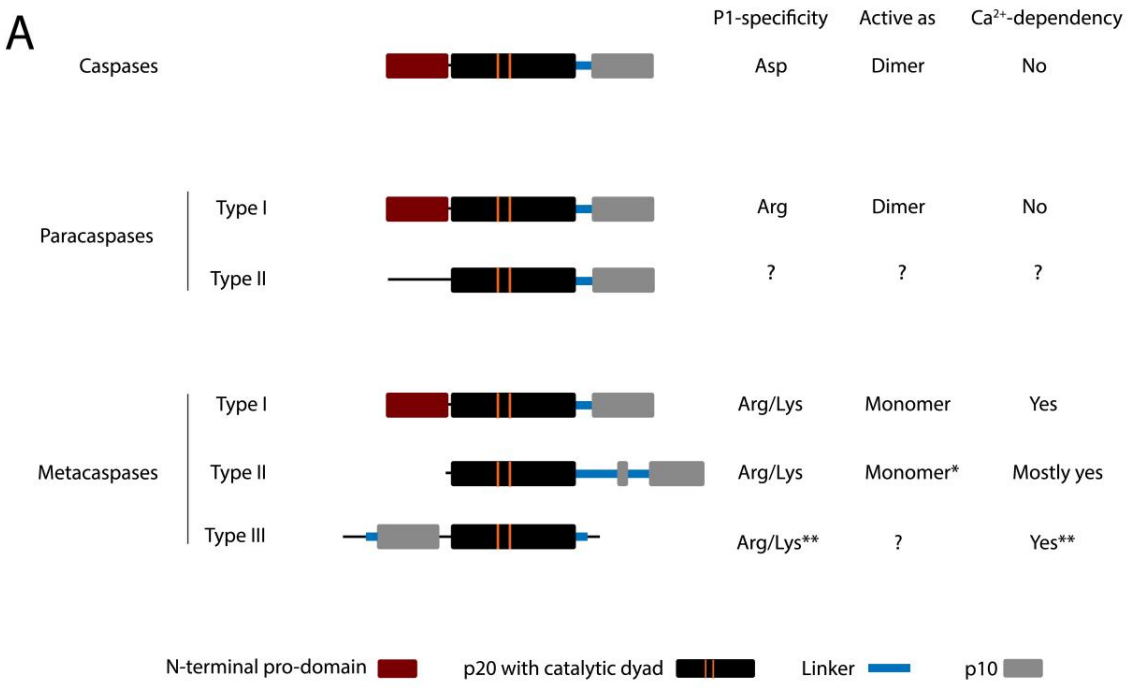

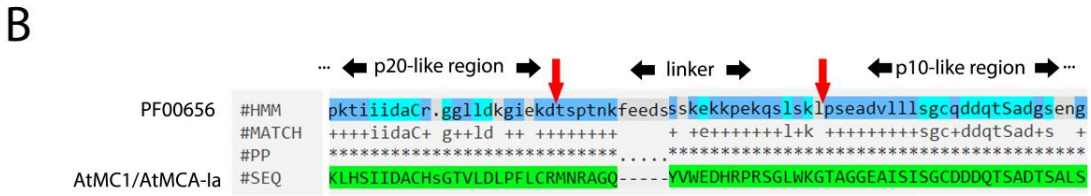

Figure 1. Classification and nomenclature of meta- and paracaspases.

(A) Comparison between caspases, metacaspases and paracaspases: domain composition and biochemical characteristics.

*Bozhkov and Smertenko, unpublished data for mcII-Pa/PaMC-IIb from *Picea abies* (Minina et al., 2017; Suarez et al., 2004).

**Only two orthologs of the type III metacaspases have been characterized so far, GtMC2/GtMC-IIIa from *Guillardia theta* (Klemenčič and Funk, 2018) and PtMC5/PtMC-IIIc from *Phaeodactylum tricornutum* (van Creveld et al., 2018).

(**B**) Part of the HMM alignment of the *Arabidopsis thaliana* metacaspase 1 with the C14 peptidase domain (PF00656), red arrows indicate borders between the p20-like region, linker and the p10-like region.

(**C**) An example of the use of the new nomenclature for the *A. thaliana* type I metacaspase. For homologs with well-established names we recommend to use the new nomenclature synonymously; this will significantly ease comparison with orthologs from species with partially sequenced genome.

Original figure

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