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

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Sciences

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spases and paracaspases: no more confusion

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Reviewer #1: This is a well reasoned, compelling, and important commentary on nomenclature of the caspases, paracaspases, and metacaspases. The authors are correct in pointing out that the names of the proteases themselves are problematic and have led to unfounded assertions and over-interpretations.
As an optional point, the authors might consider some discussion of the concept of induced proximity as it applies to caspase (and probably) paracaspase activation platforms, and whether this principle applies to metacaspases. In the context of apoptosis, induced proximity by activation platforms is the basis of the signaling pathways that orchestrate cell death; understanding when and how these can apply within the families might be helpful. Again, however, I think this is optional.

Response to reviewer:

proximity model for the activation of metacaspases, but are afraid that this interesting and important topic is beyond the scope of the classification and nomenclature document, that we have made as concise and dry as possible.

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). 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

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 (**Figure S1A**). 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

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 (**Figure S1A;** 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 (**Figure S1B**). 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 (**Figure S1A**), 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 establish a unified nomenclature of meta- and paracaspases in order (i) to facilitate comparison of orthologs from different organisms and (ii) 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 (**Figure S1C**). 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

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.

Supplemental Information

Supplemental Information includes one figure and can be found with this article online at

Acknowledgements

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S le e al I f a i

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Ele a A. Mi i a, Je S aal, Va i a E. Al a e , J h A. Be ge , Ila a Be a -F a k, R di Be ae , Ka D. Bidle, F d ic B a ci , Magali Ca a a, J a J. Ca l , Cha g Jae Ch i, N ia S. C ll, Vi h a M. Di i , Ma k D li a , Nic la Fa el, Ch i ia e F k, Pa ick Gall i , K i Ge ae , E ili G ie e -Bel a , S e ha Hailfi ge , Ma i a V E ge e V. K i , Da iel K a a , A a Li , Ma ci F. M. Machad , F a k Made , L A. Mege e , Pa agi i N. M ch , Je e C. M a , Th a N , Hei D. O ie ac , Ch i he M. O e all, Kaila h C. Pa de , J ge R la d, G S. Sal e e , Yig g Shi, A d ei S e e k , Si S ael, Je S hlbe g, Ma a Fe a da S e , Ma g Th e, Ha ele T i e , F a k Va B e ege , Re ie A. L. a de H , A af Va di, B i Zhi k , E ic La , Pe e V. B hk

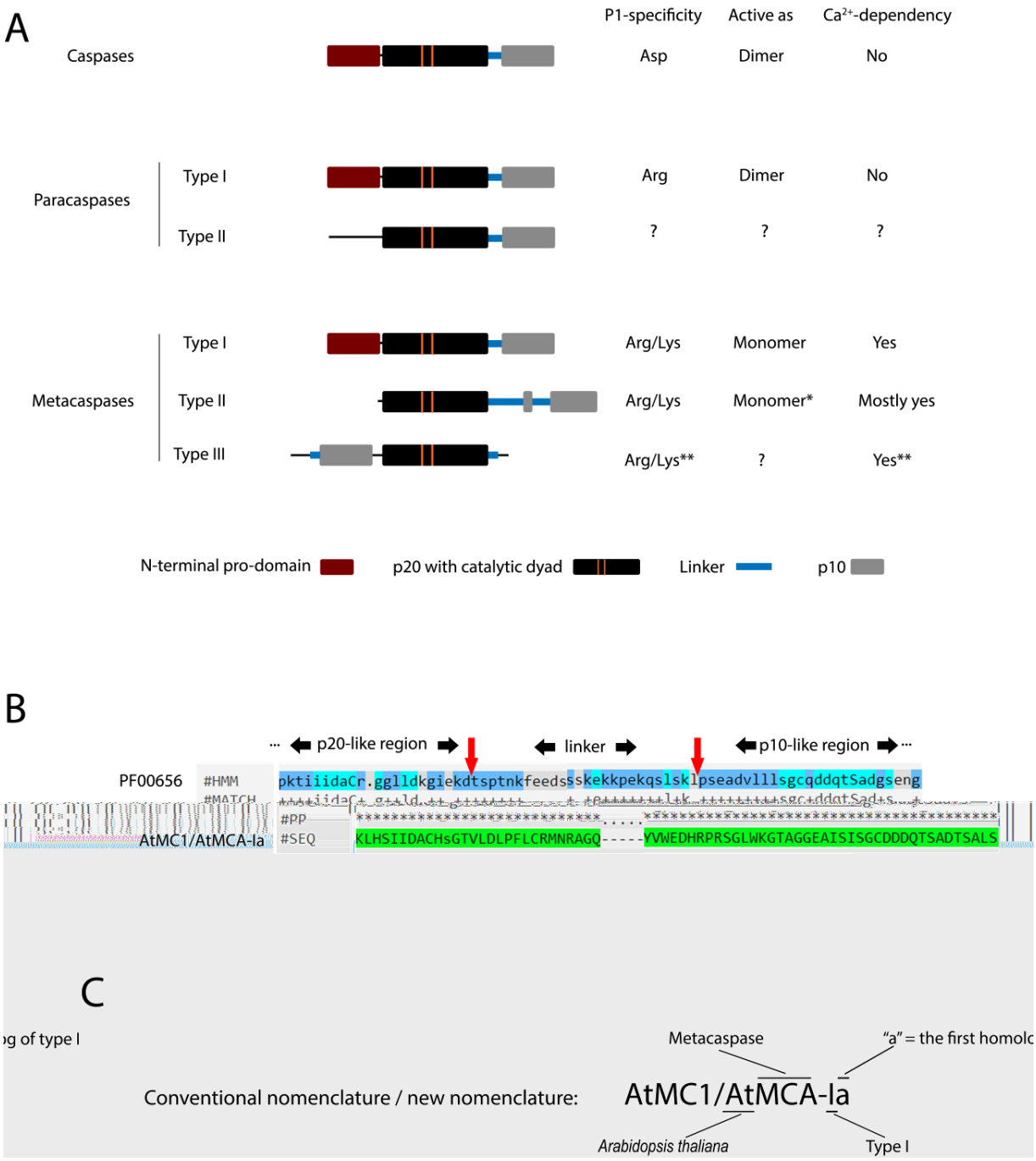

Fig e S1. Cla ifica i a d e cla e f e a- a d a aca a e .
(A) Comparison between caspases, metacaspases and paracaspases: domain composition and biochemical characteristics. *Bozhkov and Smertenko, unpublished data for mcII-Pa/PaMCA-IIb from *Picea abie* (Minina et al., 2017; Suarez et al., 2004). **Only two orthologs of the type III metacaspases have been characterized so far, GtMC2/GtMCA-IIIa from *G illa dia he a* (Klemenčič and Funk, 2018) and PtMC5/PtMCA-IIIc from *Phae dac l ic* (van

Creveld et al., 2018). **(B)** Part of the HMM alignment of the *A abid i ha*ł*ia a* 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. ha*ł*ia a* 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.

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Supplemental Figure S1 as a separate tif file

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