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Plant and animal calpain functions, association with microtubules and possible medical applications

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ABSTRACT

Calpains are calcium-activated cysteine proteases that activate a vast variety of substrates by cleavage. First reported in 1964, calpains are found in both prokaryotes and eukaryotes and display disparate multidomain architectures. The term calpainopathy was coined in 1995 when calpains were first linked to cell cycle control and cancer, and since then calpains have been implicated in many additional medical conditions including heart disease, multiple sclerosis, diabetes, sickle cell disease, and various neurological disorders. The evolution of calpains is under active investigation, but the core CysPc cysteine protease domain can be traced back to bacteria and the membrane associated MIT domain to Archaea. Here we review calpain evolution and suggest that the MIT-CysPc domain was present in the first eukaryotic common ancestor, and that this diverged into a minimum of four independent last eukaryotic common ancestors making up diverse groups from animals to land and marine plants. How calpains function at the cellular level is likewise not fully resolved. However, they are recognized to play roles in cell division, adhesion, fusion, proliferation, migration and signaling in animals and to act on stem cell functions via microtubules in land plants. Just recently calpains have also been connected to the microtubule organizing center in land plants and brown algae. We present a possible basic function of calpain domains, their connections to membranes and a possible calcium channel, supported by an updated phylogeny. Finally, we provide an overview of human calpains, potential functions and medical conditions to which they are linked and suggest possible development of calpain transcriptomic diagnostics to increase medical precision and treatment. We believe that understanding calpains has promising medical spinoffs and look forward to seeing this field unfold in the years to come.

Keywords: Calpain, calcium, cell division, microtubule, disease, cancer

Introduction

Calpains are calcium-activated cysteine proteases that alter the functions of their numerous substrates by cleavage. The name calpain combines 'cal' and 'pain', which are derived from calcium and cysteine proteases such as papain and legmain.[1] The calpain system is one of four cellular proteolytic systems along with the proteasome, lysosome and caspase systems and is involved in many cellular processes in multicellular eukaryotes.[2,3] The 15 human calpains are involved in many of our most common and serious diseases such as cancer and stem cell functions by regulating the cell cycle, neural functions linked to dementia, and oxygen transport linked to sickle cell disease.[4] Most research on calpains has been done on animals and within medical science, yet our understanding of their biological functions is still only rudimentary.

Members of the calpain family are multi-domain proteins that share a conserved core cysteine protease (CysPc) catalytic domain as well as diverse combinations of additional domains. Mammalian calpains are divided into classical and non-classical calpains based on their domain organization.[3] The nine classical calpains, represented by calpain-1 and calpain-2, are composed of a larger and a smaller subunit (**Table 1**). The larger subunit is comprised of an N-terminal anchor helix, a CysPc domain, a calpain-type beta-sandwich (CBSW) domain and a penta-EF-hand (PEF) domain. The smaller subunit consists of only a PEF domain and a glycine-rich (GR) hydrophobic region. Following activation by calcium ions, these subunits assemble to form a functional heterodimer with catalytic activity.[5,6] The six non-classical calpains consist of a single large subunit and typically function as monomers (**Table 1**). These calpains lack the PEF domain and in some cases the CBSW domain. Other domains found in various mammalian calpains include a microtubule interacting and transport motif (MIT), a C2 or C2-like (C2) domain, and a zinc-finger (Zn) motif.[3]

The calpain gene superfamily is evolutionarily ancient and its members are widely distributed among both prokaryotic and eukaryotic lineages (**Fig 1; Fig 2**). Sequences encoding the core CysPc domain are present in eubacteria, including cyanobacteria, but have not been identified in Archaea.[7,8] Genes encoding multiple calpain family members have also been cataloged in fungi, invertebrates, and mammals,[9] in unicellular eukaryotes such as *Thecamonas trahens* and *Tetrahymena thermophila*, and in several macroalgal species.[10] In contrast, land plants have one unique calpain gene, named *DEFECTIVE KERNEL1 (DEK1)*.[10,11] Interestingly, the genome of the brown macroalga *Saccharina latissima* contains sequences similar both to the land plant *DEK1* gene as well as to many of the calpain versions found in animals. (Evju et al. submitted)

Table 1. Overview of the human nine classical and six non-classical CAPN genes, expression and suggested functions. Classical calpains have a Ca^{2+} mediated conformational switch.

Calpain Gene Number	Suggested Functions	References
CAPN 1&2: Classical **Expressed:** Ubiquitously	Membrane fusion, platelet activation, cell cycle progression, cytoskeletal remodeling, cleavage of receptors, neurons functions, cell adhesion, retinal apoptosis. Calpain-1 protects while -2 is neurodegenerative, where they balance each other's brain function affecting memory.	45-47,50
CAPN 2: Classical	Age added effects of UV-damage, high sugar levels if diabetic, environmental toxins and more. Can compromise membrane proteins possibly through increased ion permeability in lens fiber cells. Embryo development.	7,54
CAPN 3: Classical **Expressed:** Skeletal muscle	Expressed in skeletal muscle, muscular dystrophy, nuclear localization. Role in Ca^{2+} release independent of its protease activity. Forms homodimers and trimers.	3,55,56,59
CAPN 5 & 6: Non-classical **Expressed:** Ubiquitously	Homologue of C. elegans sex determining gene TRA-3, exp most tissues, but especially in the central nervous system. Has 3 extended loops possibly explaining the need for higher Ca^{2+} levels for activation.	3,61
CAPN 7: Non-classical **Expressed:** Ubiquitously	Divergent sequence, more related to the fungal calpain *Aspergillus nidulans*.	62
CAPN 8&9: Classical **Expressed:** Gastrointestinal tract	Form heterodimers, CAPN8 can also form homodimers, SNPs known to inactivate them can be used as diagnostics. CAPN9 forms heterodimer with CAPN1. G1 cell cycle arrest and caspase-mediated apoptosis, tumor suppressing role by degradation tract-specific oncogenes.	44,60
CAPN 10: Non-classical **Expressed:** Ubiquitously	Insulin-mediated glucose turnover, cellular apoptosis, renal cell viability, tubule repair in renal cells. Regulator of glucose metabolism, thereby associated with development of type 2 diabetes. Requires special intracellular localization or interacting partner(s) to acquire proteolytic activity and cleaved by calpain-2.	36,52,69,70,86,87
CAPN 11: Classical **Expressed:** Testis	Expressed in testis from 14 days after birth during pachytene spermatocyte development, suggested function during meiosis.	88
CAPN12-14: Classical	**Expressed:** Hair follicle cells, ubiq and gastrointestinal Unknown functions.	
CAPN 15 & 16: Non-classical	**Expressed:** Ubiquitously, unknown functions.	

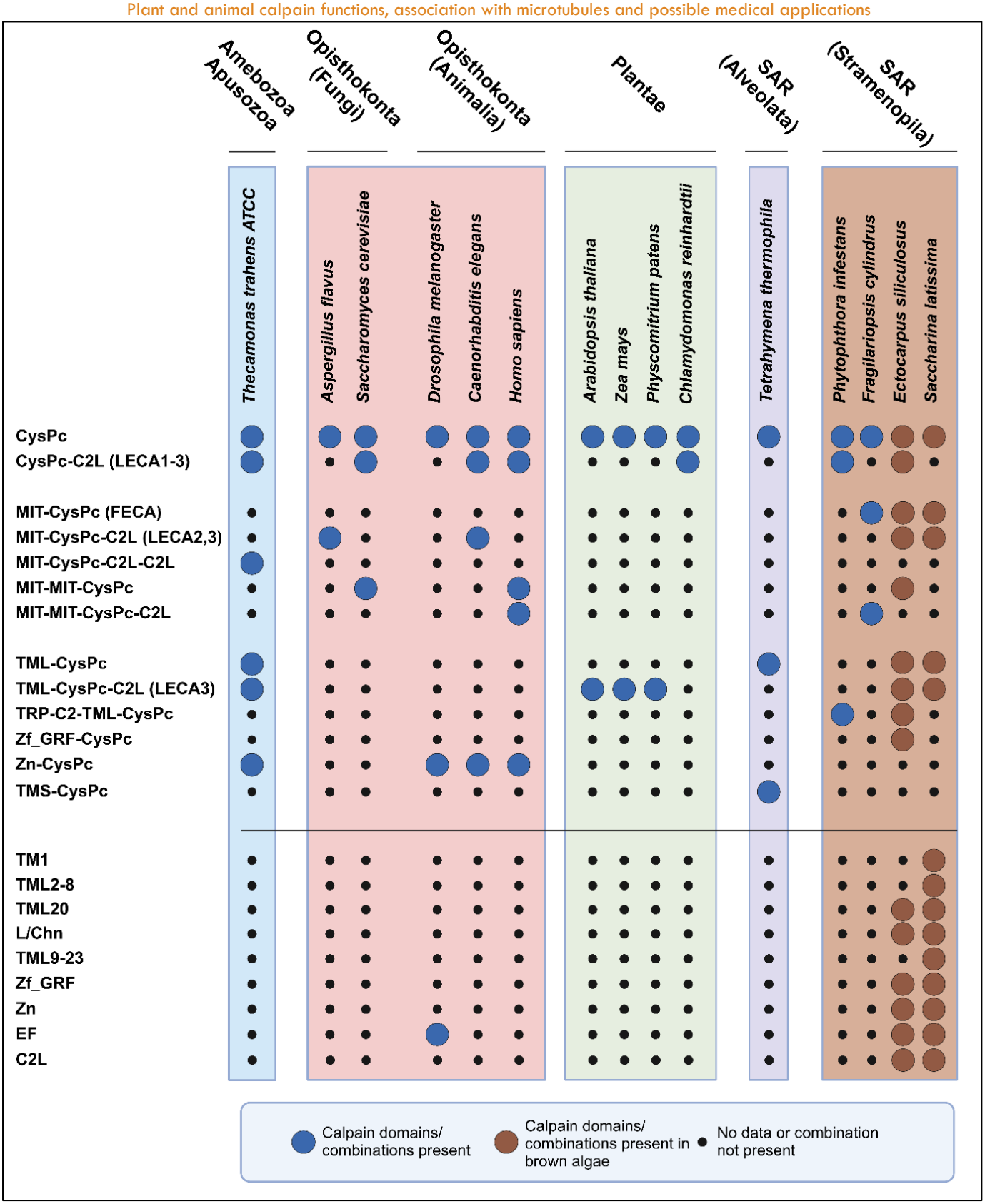

Figure 1. Calpain domain combinations detected across selected species.

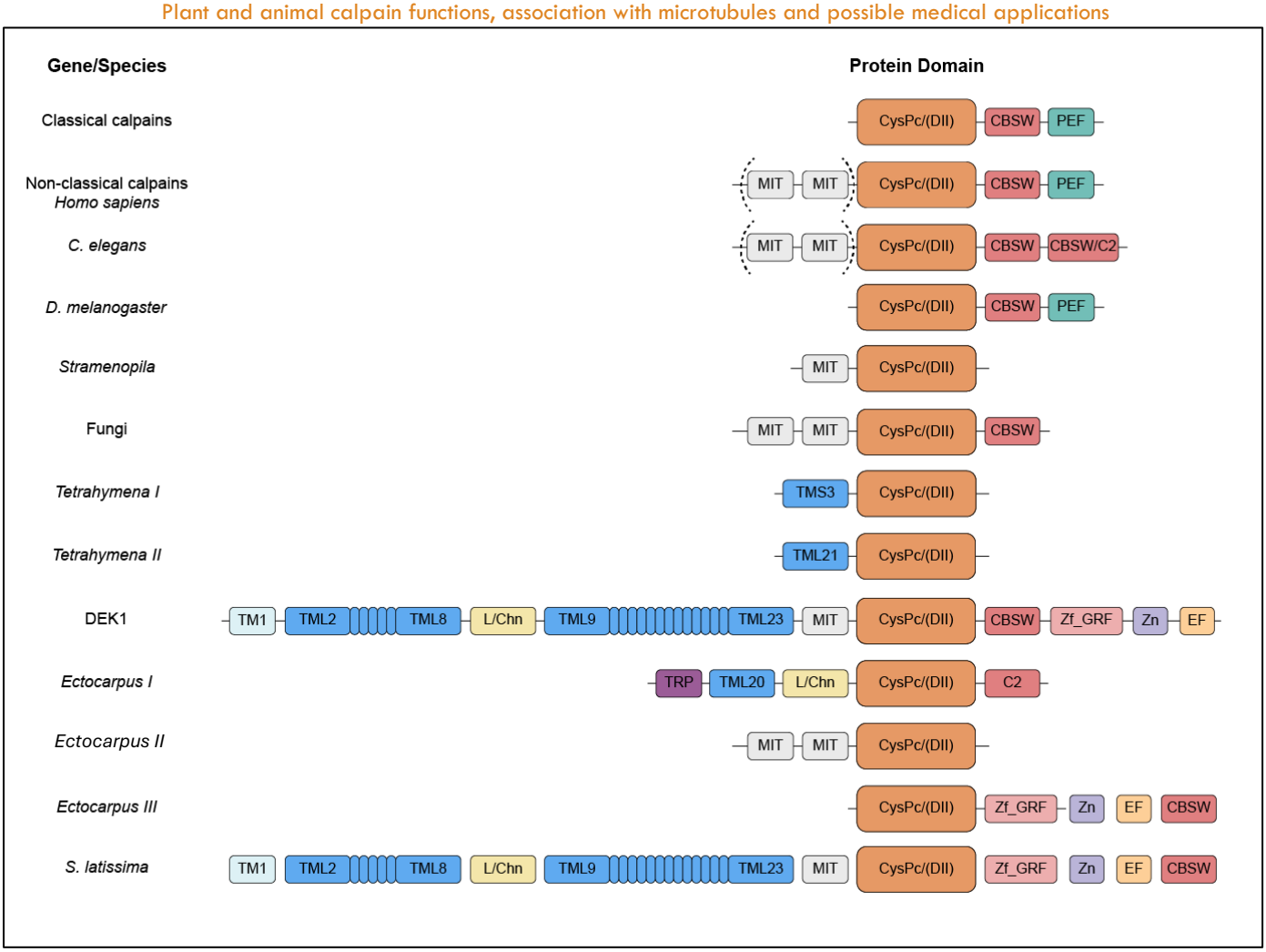

Figure 2. Schematic illustration of calpain protein domains and domain combinations across species compared to the unique plant phytocalpain protein DEK1.

The aim of this article is to review the evolutionary origin of calpains, their biological functions in land plants and animals, and their association with human medical conditions and diseases. We present an overview of recent progress in understanding calpain activity from previous studies in animals and land plants, as well as new discoveries in macroalgae that suggest biological similarities to both animal and plant calpains at the evolutionary and functional levels.[10,12] Based on these comparisons we find increased support for a core calpain function in orchestrating microtubule orientation within cells. Finally we discuss the roles of calpains in human cell biology and disease, and suggest that consideration of calpain functions in diverse species can add valuable understanding to improve medical applications of calpain-based therapies.[13]

Evolutionary origin of calpains

Four ancestral calpains date more than a billion years back.[14] Extensive phylogenetic studies of calpains seeking their evolutionary origin and possible functional implications suggest the cysteine protease domain originates from bacteria, and further that the large variation in domain architecture between and within eukaryotic species descends from four original versions early in eukaryotic history.[10] These were likely present before the split of the eukaryotic supergroups, the 1) Apusonomidae, containing Thecamonas, 2) Opistokonta, containing fungi and animals, 3) SAR, containing Tetrahymena and the brown algae Ectocarpus and Saccharina, and 4) Plantae, containing land plants (**Fig 3**). The unicellular eukaryotes from which these different supergroups evolved underwent a massive expansion of the four original calpains into 41 different domain combinations.[10] This extensive structural variation most likely arose from a combination of duplications, domain shuffling and secondary domain loss or modification during the course of evolutionary selection.[10]

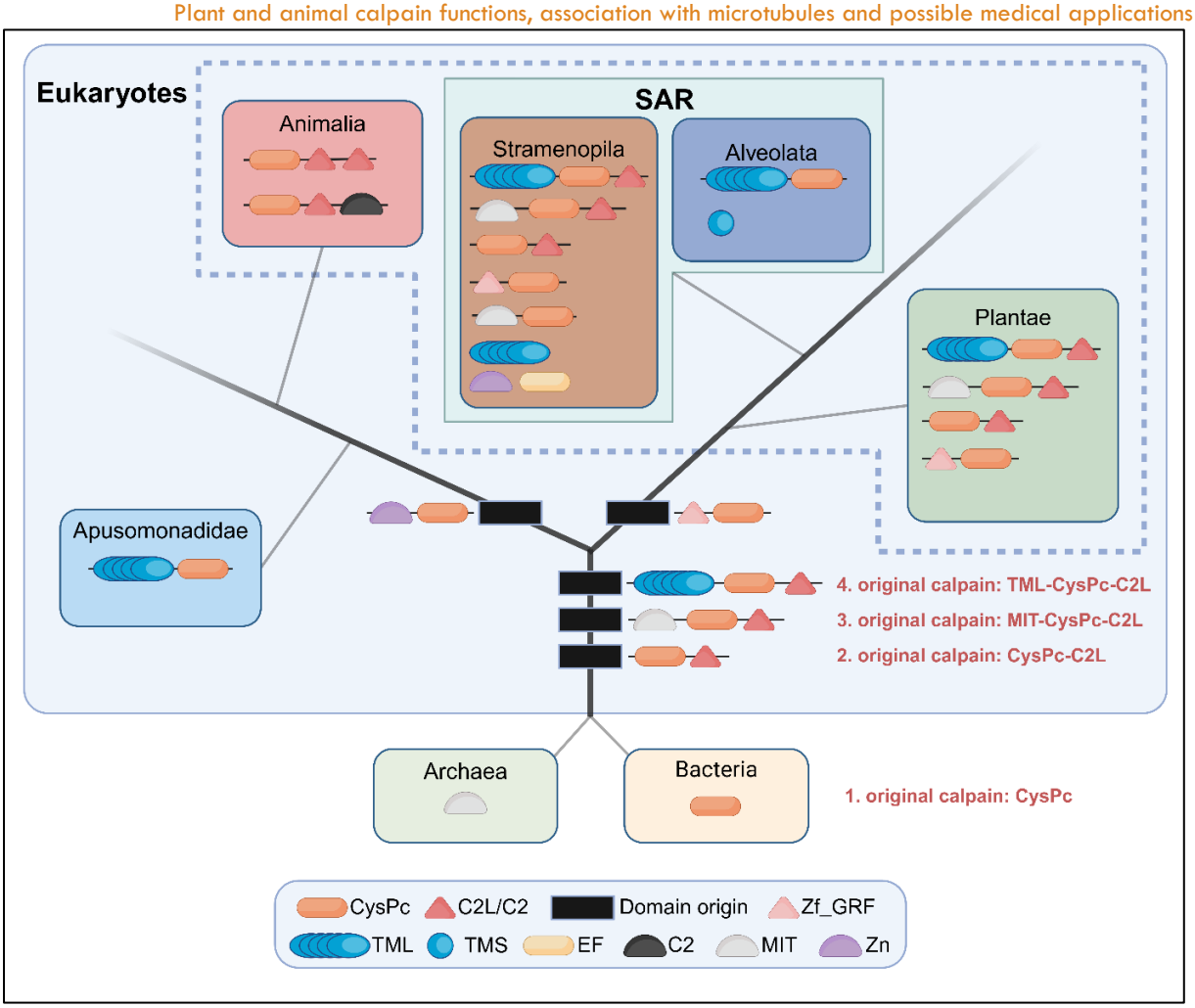

Figure 3. Tree-of-life schematic illustrating the possible evolution of calpain sequences across different supergroups of eukaryotes.

The majority of calpains are cytosolic proteins, including all those present in insects and vertebrates. These cysteine proteases function in the cytoplasm and are not anchored to cell membranes via transmembrane domains, although membrane association is linked to the activation of cytosolic calpains including the human calpains.[15,16] Similarly, although the Drosophila CALPA does not contain a defined membrane anchor domain, it is suggested to be anchored to the membrane and to depend on autolysis release for activation.[17]

Four types of membrane-anchored calpains have also been suggested, two that display broad distribution among unicellular protists and streptophytes and two that are present in oomycetes and brown algae. [16] The land plant DEK1 calpain contains a 23 transmembrane long (TML) motif interrupted by a loop or channel region (L/Chn) at its N-terminus, followed by an internal linker to the C-terminal cytosolic CysPc and CBSW domains shared with mammalian calpains.[18] Interestingly both a short 3-TM and a long 21-TM domain calpain version is found in the freshwater unicellular eukaryote Tetrahymena, an organism that has two different nuclei, cilia and flagella, the MT motor dynein and ciliary membranelles.[19] The presence in Tetrahymena of two distinct calpains containing TM domains might suggest the original evolutionary importance of the TM region to nucleic membranes, cilia and flagella functions and dynein that have been changed over time in different eukaryotic supergroups.

The membrane anchoring activity of the DEK1 calpain might have been retained in land plants due to their cell walls, a physical barrier not found in animals, possibly meaning linking directly to the membranes is not as important in organisms without cell walls. It is still striking how The camonas and metazoans have MIT suggested functioning on the cytoskeleton and possibly also connecting the cytosolic calpains to membranes, while the Tetrahymena and land plant SAR lack MIT. This might possibly explain why TML anchoring to the membranes is important in land plants, a function replacing the MIT domain in other organisms. Both are consistent with central functions by the cytoskeleton for calpain function via the MIT or TML. Thecamonas and brown algae are so far the only eukaryotes found to have retained both the MIT and TML domains, possibly allowing some branches to have kept the MIT while plants kept the TML.[10] The TML anchors the calpain to the cell membranes, such as the outer cell membrane and nuclear envelope. Calpain membrane activity has only been studied in land plants and might give us insight into the possible basic function of calpains, including those in animals.

We suggest there existed three phylogenetically distinct membrane-bound calpains in the LECAs, now present in land plants, brown algae and animals as presented in **Figure 3.**[14,16] The membrane-associated land plant calpain, DEK1, is activated by Ca^{2+} in the presence of an experimentally added channel.[20] The substantial membrane part of DEK1 has also been suggested to possibly contain a Ca^{2+} channel region within it, potentially explaining why the membrane anchor has

been retained in this calpain through evolution.[13] DEK1 is indicated to be functionally involved in regulating the internal, cytosolic portion of its calpain through a Ca^{2+} channel further linked to the organization of MT.[13,20] The brown macroalga *Ectocarpus* and *Saccharina* both contain sequences similar to the transmembrane domains of the land plant DEK1 calpain as well as to many of the calpain versions found in animals (**Fig 1; Fig 2**),[10,Evju et al. submitted] suggesting that macroalgal calpain proteins may be composites in which both animal- and plant-associated domains were retained.

In animals the cytosolic calpains, although not bound to membranes, are consistently reported to be associated with them.[15] The animal calpain-1 and calpain-2 proteins are bound to Transient Receptor Potential (TRP) channels and even activate TRPC5.[16,21] The TRP channels make up a superfamily of Ca^{2+} permeable nonselective membrane cation channels and contribute to intracellular Ca^{2+} fluxes and cell signaling.[22,23] These channels often form units of 24 transmembrane pores specific to eukaryotes.[24,25] TRP channels have not been identified in land plants but are present in the closely related green algae,[22] opposite to DEK1, suggesting that TRP channel activity might replace DEK1 function in green algae.

Functions of plant calpains to set 3D cell orientation via microtubules

The unique land plant calpain gene *DEK1* was first identified from a maize *(Zea mays)* mutant collection having aborted seeds, shown to be due to loss of epidermal cells and embryo arrest at the globular stage.[11,26] We further found the gene to be expressed in most cells, with higher expression in actively dividing cells and in stem cells in particular.[13,27] Arabidopsis *dek1* mutant embryos have defective cell division patterns and planes, and in more severely affected embryos, nearly all the cell walls are incorrectly positioned.[13] These defects are associated with disorganized arrangements of microtubules (MTs) within the cells, demonstrating that DEK1 regulates the arrangement of the cortical MT systems during early embryo development, which in turn may affect cell wall deposition. Together the data suggest that DEK1 sets the 3D cell division orientation for plant embryos to progress beyond globular stage by positioning cell walls according to their microtubule orientation.[13,28]

DEK1 also affects the cell divisions in the developing suspensor. In land plants the upper tier of the dividing zygote develops into the embryo proper, while the lower tier forms an extra-embryonic suspensor structure connecting it to the surrounding endosperm tissues. The suspensor divides exclusively through anticlinal cell divisions before dying by programmed cell death, whereas the embryo proper divides in various planes to set the 3D orientation developing the new plant generation. In Arabidopsis *dek1* mutants, the suspensor develops like the embryo proper by dividing in the periclinal orientation and expressing genes otherwise only present in the apical embryo. This suggest repressive signals from the embryo proper are needed to keep the suspensor from carrying out its embryogenic potential.[13,29] Based on the *dek1* phenotypes, DEK1 is proposed to restrict the basal development of the suspensor by preventing periclinal cell divisions and regulating the position of embryogenic marker genes.[13] One such marker is *WOX2,* the expression of which in the apical cells depends on a first asymmetric cell division of the zygote. *dek1* mutants fail to express *WOX2*, likely due to a failure of the microtubule-orientated asymmetric first cell division. In addition, expression of the hormone transport protein *PIN4*, which is normally restricted to the suspensor, occurs ectopically in the *dek1* embryo proper.[13] The lack of proper PIN4 protein localization might be a factor in why *dek1* mutants are unable to repress proper embryo development.[30,31]

Medical science can achieve important insights from comparing the functions of evolutionary-related genes in different species. *DEK1* is an ideal candidate to study calpain activity, because it occurs as a single copy in plants whereas multigene calpain families and the many varying sequences in other important eukaryotes complicate functional studies. To date the plant studies are consistent with a fundamental role for DEK1 in directing asymmetric cell divisions, in particular when shifting from 2D to 3D orientations, and in activating the expression of genes that depend on asymmetric divisions for their spatial patterning.[13,32,Evju et al. submitted] The requirement of DEK1 for correct orientation of cell divisions is associated with a function in regulating MT organization, indicating a core role for DEK1 in cytoskeletal control. We suggest DEK1 acts by organizing the MT and argue this could be a core function of calpains in animals also. Animals have both centrosomes and microtubule organizing centres (MTOC), as do brown algae, whereas land plants lack centrosomes and therefore depend on the MTOC to organize the cytoskeleton throughout the cell cycle and to control cell division.

Calpain association with medical conditions - utility in diagnostics and therapy

In animals calpains have been linked to a wide spectrum of functions including calcium regulation, signal transduction, cytoskeleton dynamics, cell mobility, cell cycle progression, long-term potentiation in neurons, muscle protein break-down, cell adhesion, cell fusion and apoptosis.[33-36] Calpains are known to be involved in pathophysiological mechanisms and specifically in human medical conditions such as cancer,[37] Alzheimer's disease,[38,39] multiple sclerosis, Parkinson's disease,[40] Huntington's disease,[41] Type2 diabetes, estrogen-mediated cancer metastasis, aging related syndromes, sickle cell disease, asthma,[42,43] cardiac dysfunction and blindness,[36] and even alcoholism and malaria.[44] These pathological functions are associated with either reduced or elevated calpain activity, leading to decreased or excessive substrate cleavage, respectively.

The classical calpains are numbered calpain-1, 2, 3, 8, 9, 11, 12, 13 and 14 (**Table 1**). Calpain-1 and calpain-2 function in membrane fusion, platelet activation and cell cycle progression,[45,46] cytoskeletal remodeling, and cleavage of receptors such as epidermal growth factor (EGF) in neurons and the eye especially.[47] Calpains have been shown to reduce function in retinal apoptosis, by downregulating proapoptotic proteins and NF-kappaB

and thereby neuroprotecting retinal ganglion cells.[48] Together with caspase-3 they can cleave cytoskeletal, cytosolic and nuclear substrates.[49] Calpain-1, calpain-2 and caspase-3 further have synergistic effects on acute neuronal cell death, while surprisingly Calpain 1 and 2 have opposite effects. Calpain-1 is neuroprotective while calpain 2 is degenerative, suggesting downregulation of calpain-2 may be a target for neural degenerations including traumatic brain injury and repeated concussions.[50] Calpain inhibition has further been shown to slow down cataract formation by protecting cytoskeletal proteins from calpain proteolysis.[51]

Calpain-1 and -2 are linked to neurological disorders – stroke and Alzheimer's disease (AD) – as well as several types of cancer.[52] They are found to be upregulated in breast cancer cells and to suppress cancer treatment effects, but this differs between cancer types and calpains (**Table 2** and see below). Knockout of *CAPN1* also leads to neurological disorder spastic paraplegia 76.[47] In addition, Calpain-1 and -2 have been shown to cause disassociation of tau from MT, thereby leading to neuronal death, making them targets for treatment of Alzheimer's disease.[53] Calpain-2 is reported to cause added effects of UV-damage, high sugar levels if diabetic, environmental toxins and to compromise membrane proteins possibly through increased ion permeability in lens fiber cells.[54] In mice, calpain-2 is also needed for embryo development.[7]

CAPN3 is expressed in skeletal muscle (**Table 1**), and the nuclear-localized protein is linked to muscular dystrophy.[55,56] Calpain-3 is linked to Limb-girdle muscular dystrophy (LGMD) – named calpainopathy and is also the first calpain linked to cancer. *CAPN3* is highly expressed in melanoma cell lines and bovine bladder tumors, and induces increased stabilization of p53 and increased reactive oxygen species production leading to reduced cell proliferation and increased cell death.[57] This led the authors to suggest that reduced *CAPN3* expression contributes to melanoma progression, with both diagnostic and therapeutic options. Conversely, upregulation of *CAPN3* is proposed to lead to overexpression of *E2F3*, in turn causing urothelial tumor cell proliferation.[58] Interestingly calpain-3 has been found to have a role in Ca^{2+} release independent of its protease activity.[59]

Table 2. Overview of human calpain medical associations.

Calpain Number	Medical Association	References
Calpain-1	Neurological disorders such as stroke, Alzheimer's disease (AD), spastic paraplegia 76.	47,52
Calpain-2	Transcript marker of sudden cardiac death and a potential therapeutic target in various forms of neurodegeneration, including traumatic brain injury and repeated concussions.	50,75
Calpain-1&2	Upregulated in breast cancer cells and suppresses cancer treatment effects. Cause disassociation of tau from MT – neuronal death therefore targets for AD treatment. Calpain inhibition has been shown to slow down cataract formation in humans.	
Calpain-3	Limb-girdle muscular dystrophy (LGMD). Highly expressed in melanoma cell lines and bovine bladder tumors.	55,89,90
Calpain-5	ADNIV.	91
Calpain-9	Gastric cancer, suppression of tumorigenesis.	60
Calpain-10	Type 2 diabetes (T2D).	52,68,88,92
Calpain-11 Calpain-12	Functions during meiosis for sperm function. Developmental eye disorders caused by disruption of the optic fissure disclosure.	93
Calpain-14	Eosinophilic esophagitis.	94

CAPN8 and *CAPN9* are expressed in the gastrointestinal (GI) tract (**Table 1**), can be inactivated by specific SNPs, form dimers with each other and can be used as diagnostics for gastric cancer.[44] Calpain-9 is linked to G1 cell cycle arrest and caspase-mediated apoptosis[60] and is suggested to have a tumor suppressing role by degrading GI tract-specific oncogenes. *CAPN11* is expressed in testis, whereas *CAPN12* is expressed in hair follicles (**Table 1**).[44]

Among the non-classical calpains, human *CAPN5* is homologous to the C. *elegans* sex determining gene *TRA-3* and expressed in most tissues, but especially in the central nervous system.[44] *CAPN6* is highly homologous to *CAPN5*, is located on the X chromosome and is also suggested to be involved in sex determination.[61] *CAPN7* has a more divergent calpain sequence making it easier to design a calpain specific antigene to this member, and the divergence includes the MIT-MIT, and this calpain sequence is more similar to the fungal calpain *Aspergillus nidulans*.[62] It regulates timing and completion of abscission by both the tandem MIT distinct motifs to complete cytokinesis, secure checkpoint maintenance and separate dividing cells.[63] Calpain-7 function on the Endosomal Sorting Complexes Required for Transport (ESCRT) is thereby required for the membrane fission step to complete cytokinesis and separation of cells. The human *CAPN10* gene is most highly expressed in the heart, followed by the pancreas, brain, liver and kidney (**Table 1**).[52]

A subset of the non-canonical calpains are associated with various types of diseases and cancers (**Table 2**). Calpain-5 can cause the rare ocular autoimmune disorder Autosomal Dominant Neovascular Inflammatory Vitreoretinopathy (ADNIV). *CAPN6* has increased expression in uterine cancers, whereas *CAPN9* has reduced expression in gastric cancer and is suggested to cause gastric cancer and suppression of tumorigenesis.[64,65] Calpain-10 is linked to colorectal

cancer,[66] pancreatic cancer[67] and type 2 diabetes.[36,52,68-70] Mutated *CAPN10* gene versions elicit a reduced rate of insulin-mediated glucose turnover, explaining how loss of calpain-10 function leads to type 2 diabetes.[71] Increased numbers of free fatty acids in obese people leads to increased Ca^{2+} activation of calpain, leading to induces major endoplasmic reticulum stress markers causing cellular apoptosis.[36] Calpain-10 is also needed for renal cell viability and decreases with age. It is further important for tubule repair in renal cells, a cleavage of cytoskeleton proteins that may lead to increased membrane permeability causing renal cell death.[69,70]

Multiple calpains are linked to neuronal death, apoptosis, deficits of synaptic transmission, lens cytoskeletal protein cleavage and cleavage of oncogene products (**Table 1**).[72] Calpains are reported to be markers of melanoma progression such as colorectal adenocarcinomas, breast and prostate cancer (**Table 2**),[73] of tuberculosis,[74] and of sudden cardiac death,[75] and are highly expressed in melanoma cell lines and bovine bladder tumors.

Like the land plant calpain DEK1, some animal calpains are connected with Ca^{2+} channel activity, the cytoskeleton, and/or microtubule organization (**Table 1**). Calpain-1 and -2 bind to TRPC6 in mice and regulate the cytoskeleton[76] and the human calpain-1 and -2 proteins cleave and activate TRPC5 associated with neuronal growth.[21] CAPN1, CAPN2 and CAPN6 are all reported to be involved in cytoskeletal organization and CAPN5, 6, 7 and 10 are found to stabilize MT. This is all consistent with our suggested role for calpains in regulating MTOC activity in animals as in land plants. We have linked the DEK1 calpain to controlling plants MTs through the MTOC,[13] and in animals this is further associated with immunology and T-cell receptors (TCR) reorganizing of the MTOC linked to dynein, GTPases, integrins and actin.[77]

Multiple human calpains are involved in cytoskeletal remodeling (**Table 1**). Cytoskeletal connections between calpains and cancer include calpain-mediated cleavage product MYC-nick (MYC proto-oncoprotein) that promotes cytoskeletal remodeling, which is upregulated in cancer cells.[44] Myc oncoproteins are widely involved in oncogenesis, and calpain-3 cleavage generates myc-nick that induces alpha-tubulin altered cell morphology by recruiting histone GCN5 to MT, and that drives cytoplasmic reorganization and differentiation.[78] Accelerated calpain cleavage of the human epidermal growth factor receptor (HER2) has been found to repress the targeting of such cancers, since they contribute to resistance to anticancer therapies.[44,73] Further reduction of antitumor therapy effectiveness is caused by calpain-1 and -2 modifications, since they regulate the cellular efflux machineries for drug efflux.[73]

Calpains have similarly been suggested to cause cancer by reduced cell adhesion leading to cell release from the cellular matrix, including cell transformation, migration and invasion.[79] Since calpains are recognized as key regulators of cell adhesion, they could promote either epithelial cell clearance or migration through adherens junctions (AJ) and focal adhesion (FA) complexes.[80] Interestingly AJs are found crucial to epithelia identity and are further linked to the actin cytoskeleton, the establishment of polarity and cell-cell communication needed for cell proliferation and movement. RhoA and Piezo are downstream targets of FA, and are regulated by the DEK1 calpain.[81] Both calpain-1and calpain-2 are known to target actin binding proteins, and can proteolyze E-cadherin and other adhesion proteins during mammary gland development.[82] These data show the important role of calpains in cell adhesion disruption and actin dynamics, further suggesting roles not yet discovered.[80]

Interestingly the function of calpains in tumor cell migration and invasion is better understood than their role in apoptosis and cancer survival, related to cell adhesion and actin dynamics.[64] Stress is further linked to increased Ca^{2+} levels and overactive calpains causing endothelial cell dysfunction, increased cytoskeleton degradation and organ dysfunction implicated in cardiac dysfunction.[36] Downregulation of several calpains are therefore suggested as therapeutic interventions in cancer treatments[83,84]

Emerging calpain-targeted therapeutic strategies are being developed to reduce calpain activities through alternatives to calpastatin regulation, including peptidyl epoxide, aldehyde and ketoamide. However, the limiting factor is the lack of calpain specificity, pointing to selecting locations outside of the shared domain sequences.[85] Many more therapeutic options are expected as we understand calpain functions better to predict the right balance to maintain healthy cells and prevent diseases linked to calpain disfunction.[37,44]

Conclusion

Members of the calpain family of cysteine proteases are associated with a variety of severe human diseases, including cancer, multiple sclerosis and various neurological disorders. The canonical calpain cysteine protease domain arose in bacteria, followed by a massive expansion of domain structures such that extant calpain proteins consist of multiple domains in combinations that vary across the evolutionary spectrum. Correspondingly, calpains have been linked to a wide range of biological functions in plants and animals; many of these are associated with cell membranes and cytoskeletal remodeling. Such functions point to a potential core role for calpains in organizing microtubules within cells, as shown for the land plant calpain DEK1. With therapeutic strategies emerging to alter calpain activity and thus reduce cellular dysfunction, further investigation of calpain cellular and biochemical functions in animals, plants and algae can provide new directions for therapeutic intervention.

Conflicts of Interest Statement

The authors declare no conflicts of interest.

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