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SHORT COMMUNICATION

Adaptive eukaryote-to-eukaryote lateral gene transfer: stress-related genes of algal origin in the closest unicellular relatives of animals

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Abstract

In addition to mutation, gene duplication and recombination, the transfer of genetic material between unrelated species is now regarded as a potentially significant player in the shaping of extant genomes and the evolution and diversification of life. Although this is probably true for prokaryotes, the extent of such genetic exchanges in eukaryotes (especially eukaryote-to-eukaryote transfers) is more controversial and the selective advantage and evolutionary impact of such events are less documented. A laterally transferred gene could either be added to the gene complement of the recipient or replace the recipient's homologue; whereas gene replacements can be either adaptive or stochastic, gene additions are most likely adaptive. Here, we report the finding of four stress-related genes (two ascorbate peroxidase and two metacaspase genes) of algal origin in the closest unicellular relatives of animals, the choanoflagellates. At least three of these sequences represent additions to the choanoflagellate gene complement, which is consistent with these transfers being adaptive. We suggest that these laterally acquired sequences could have provided the primitive choanoflagellates with additional or more efficient means to cope with stress, especially in relation to adapting to freshwater environments and/or sessile or colonial lifestyles.

Introduction

The last decade has marked a big change in our appreciation of how genomes evolve. In addition to mutation, gene duplication and recombination, the transfer of genetic material between unrelated species is now regarded as a potentially significant player in the shaping of genomes and the evolution and diversification of life. Although this is probably true for prokaryotes, the extent and the impact of such genetic exchanges on the evolution of eukaryotes are yet to be determined (e.g. Boucher *et al.*, 2003; Andersson, 2005; Keeling & Palmer, 2008). Two types of gene transfer are generally described in eukaryotes: endosymbiotic gene transfer (EGT) and lateral or horizontal gene transfer (LGT or HGT). The transfer of genes during the establishment of long-standing intracellular symbioses with either prokaryotes (such as during the evolution of mitochondria and primary plastids) or eukaryotic photosynthetic algae (such as during the integration of secondary and tertiary plastids) is well documented and its contribution to eukaryotic evolution well established (Archibald & Keeling, 2002; Cavalier-Smith, 2002; Keeling, 2004). Nevertheless, the acquisition of foreign genes through other means (via food, viruses, host–parasite interactions) is more controversial (see Andersson, 2005 for a discussion).

Furthermore, although many examples of LGT from prokaryotes to eukaryotes have been reported (e.g. Boucher & Doolittle, 2000; Nixon *et al.*, 2002; Andersson *et al.*, 2003, 2006; Rogers *et al.*, 2007), considerably fewer instances of potential LGTs between eukaryotes are known (for reviews, see Andersson, 2005 and Keeling

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& Palmer, 2008). Lastly, although some of the prokaryote-to-eukaryote gene transfers involved prokaryotic-specific metabolic genes that are thought to confer the eukaryotic recipient the ability to adapt to a new environment, niche or lifestyle (e.g. an anaerobic or a sugar-rich environment; Andersson *et al.*, 2003; Opperdoes & Michels, 2007), in the case of eukaryote-to-eukaryote transfers, the selective advantage of such events is less documented (for a review, see Keeling & Palmer, 2008).

Generally, a laterally transferred gene could either be added to the gene complement of the recipient (gene addition), or replace the recipient's homologue (functional gene replacement). Replacement events can be adaptive or stochastic; the latter are associated with the random inactivation and loss of one of the functionally equivalent genes, and are facilitated by a gene replacement 'ratchet' mechanism (Doolittle, 1998; Stechmann *et al.*, 2006). On the other hand, gene additions are most likely adaptive. Notably, most of the eukaryote-to-eukaryote gene transfers reported to date are gene replacements (Andersson, 2005), and only a few potential cases of adaptive gene additions have been reported (for a review see, Keeling & Palmer, 2008).

To explore the possibility that gene exchanges between unrelated eukaryotic lineages could have also greatly contributed to the evolution and diversification of eukaryotes, we focused on the transfer of genes with the potential to provide the recipient with additional or enhanced adaptive capabilities. The detection of such events can be facilitated if the transferred genes are limited to a specific eukaryotic group and the donor and recipient are distantly related.

The current view of the 'eukaryotic tree' is centred around a hypothetical model composed of several large eukaryotic 'supergroups' that diverged from each other early in the evolution of eukaryotes (e.g. Keeling *et al.*, 2005). All fungi, animals and their unicellular relatives are members of the Opisthokonta – which together with the Amoebozoa are included in a strictly nonphotosynthetic assemblage of lineages, the Unikonts (Stechmannn & Cavalier-Smith, 2003). The other four eukaryotic supergroups (also known as Bikonts; Cavalier-Smith, 2003) consist of either primarily photosynthetic lineages (Plantae – i.e. glaucophytes, red and green algae and land plants) or a mixture of nonphotosynthetic and secondarily photosynthetic lineages (Chromalveolata – e.g. diatoms, ciliates, apicomplexans and oomycetes; Excavata – e.g. euglenoids, diplomonads and trypanosomatids; Rhizaria – e.g. chlorarachniophytes).

As the most significant distinction among eukaryotes is between photosynthetic and nonphotosynthetic lineages, here we focused on the transfer of genes from photosynthetic to nonphotosynthetic eukaryotes; specifically, from photosynthetic algae to the closest unicellular relatives of animals, the choanoflagellates (King, 2004). Sequence data for two unicellular choanoflagellate species have recently become available through the *Monosiga brevicollis* genome sequencing project (http://genome.jgi-psf.org/Monbr1/) at the Joint Genome Institute (JGI; http://www.jgi.doe.gov) and the *Monosiga ovata* EST project (TBestDB; http://amoebidia.bcm.umontreal.ca/pepdb/). The two *Monosiga* species inhabit distinct habitats (marine vs. freshwater), have distinct lifestyles (planktonic vs. sessile) and appear to have separated early in the evolution of the choanoflagellate lineage (Cavalier-Smith & Chao, 2003). Furthermore, phylogenetic analyses using various sequences support the inclusion of choanoflagellates, animals and fungi into Opisthokonta – to the exclusion of all photosynthetic lineages (e.g. Cavalier-Smith & Chao, 2003; Nozaki *et al.*, 2007).

What type of algal genes could provide a significant enough adaptive advantage to account for their being retained in the genome of the *Monosiga* ancestor after a random gene incorporation event? Compared with their nonphotosynthetic relatives, photosynthetic eukaryotes posses a more complex antioxidant system required to cope with the potentially damaging reactive oxygen species (ROS) released as by-products of their photosynthetic activities. Notably, increased levels of cellular ROS (oxidative stress) are also associated with environmental and biotic stress (e.g. Mittler, 2002). Thus, additional or more efficient means to deal with oxidative stress can be selectively advantageous. This is particularly true for sessile species (due to their inability to avoid environmental or biotic stressors) as well as during the colonization of new habitats; such selective pressures have been previously invoked to explain the diversification of antioxidant systems in land plants (Fink & Scandalios, 2002).

To address this possibility, we searched the two *Monosiga* databases for sequences coding for proteins known to have antioxidant activities and/or be involved in various cellular responses to stress (e.g. ROS and redox metabolism, defence and cell death). Several *M. brevicollis* sequences coding for stress-related proteins (e.g. glutathione and ascorbate peroxidases, heat shock Hsp20 and universal stress proteins, glutaredoxin and thioredoxin reductase, metacaspases) with Blast best hits among plant and algal homologues were found. We focused on those that: (i) were strongly supported by multiple types of evidence – including phylogenetic distribution, domain organization, specific insertions and sequence affiliations; and (ii) were most likely to represent adaptive gene transfers. Here, we report four stress-related sequences (two ascorbate peroxidase and two metacaspase genes) of algal origin in the closest unicellular relatives of animals; at least three of these sequences represent additions to the choanoflagellate gene complement, which is consistent with these transfers being adaptive. We suggest that these eukaryote-to-eukaryote gene transfers could have contributed to the evolution and diversification of the choanoflagellate lineage.

Materials and methods

The *Monosiga* genome database (http://genome.jgi-psf.org/Monbr1/) was searched for stress-related sequences via the GO, KEGG, KOG, Advanced Search and BLAST options. The other sequences used in this study were retrieved from JGI (http://www.jgi.doe.gov), Interpro (http://www.ebi.ac.uk/interpro/), GenBank (http://www.ncbi.nlm.nih.gov/), the TbEST database (http://amoebidia.bcm.umontreal.ca/pepdb) and several other genome databases (e.g. http://merolae.biol.s.u-tokyo.ac.jp/; http://genomics.msu.edu/galdieria/), using text and Blast (tblastn and blastp) searches (Altschul *et al.*, 1990). All sequences were checked for the presence of functional domains using SMART, InterProScan and Pfam (http://smart.embl-heidelberg.de/; http://www.ebi.ac.uk/InterProScan/; http://www.sanger.ac.uk/Software/Pfam/search.shtml) and aligned with Muscle (http://www.drive5.com/muscle/; (Edgar, 2004). Phylogenetic analyses were performed using MrBayes v3.0B4 (mixed amino acid model; 3 500 000 generations; 100 sample frequency; 5000 burnin) and PhyML (http://atgc.lirmm.fr/phyml/; 200 replicates; four-category gamma distribution; proportion of variable sites estimated from the data; best-fit amino acid model indicated by ProtTest) (Huelsenbeck & Ronquist, 2001; Abascal *et al.*, 2005; Guindon *et al.*, 2005). TargetP (http://www.cbs.dtu.dk/services/TargetP/; Emanuelsson *et al.*, 2000) was used to predict cellular localizations.

Results and discussion

Ascorbate peroxidases

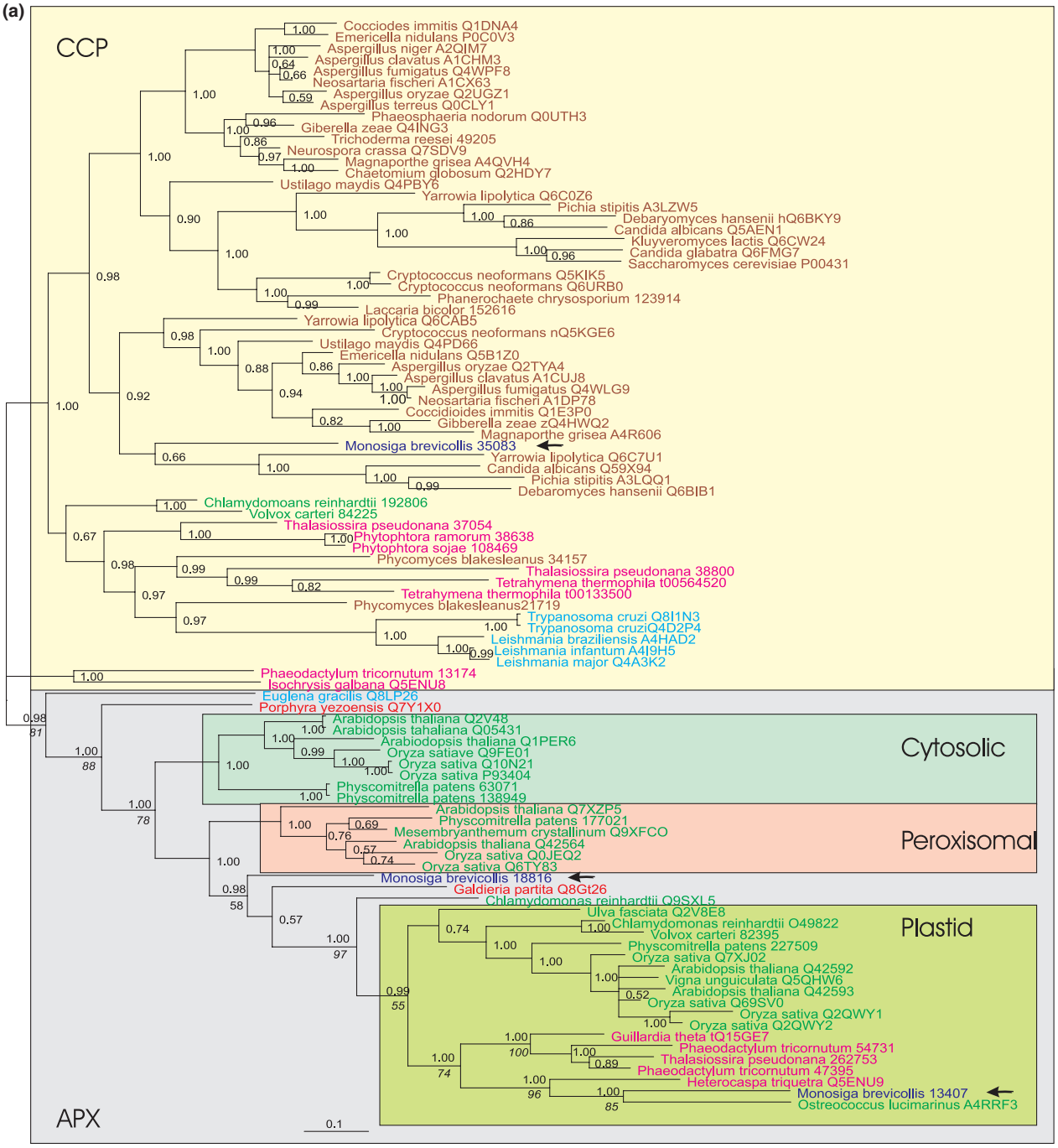
Several antioxidant systems are known in photosynthetic organisms. Ascorbate peroxidases (APXs) belong to a large superfamily of 'nonanimal peroxidases' – namely, the plant/fungal/bacterial peroxidases; specifically, APXs are members of the Class I peroxidases that also includes cytochrome *c* peroxidases (CCPs) and bacterial catalase peroxidases (Passardi *et al.*, 2007). CCPs and APXs contain the plant ascorbate peroxidase domain (IPR002207), are closely related and are thought to share a common ancestry (Passardi *et al.*, 2007). However, although CCPs are present in both photosynthetic and nonphotosynthetic eukaryotes (although missing in some protists, animals and land plants), APXs have only been found in plastid-containing lineages, both primarily photosynthetic (i.e. glaucophytes, green algae, red algae and land plants) and secondarily photosynthetic (e.g. diatoms, *Euglena*) lineages (Teixeira *et al.*, 2004; Passardi *et al.*, 2007).

Our search for proteins containing the plant ascorbate peroxidase domain in the genome of *M. brevicollis* (Mb) identified three such sequences (protein IDs: 35083, 13407 and 18816). Phylogenetic analyses indicate that, while Mb35083 affiliates with fungal mitochondrial CCPs, Mb13407 and Mb18816 group within the APX clade (Fig. 1a). The affiliation of Mb35083 with fungal mitochondrial CCPs is expected and is consistent with the sequence being inherited from the last common ancestor of fungi and choanoflagellates; its predicted mitochondrial localization further supports this affiliation. However, the branching of Mb13407 and Mb18816 sequences within the APX clade requires a different explanation. As APX sequences are absent from bacteria and have only been found in plastid-containing photosynthetic lineages (Passardi *et al.*, 2007), the most parsimonious scenario entails the acquisition of the two *Monosiga* APX sequences from a eukaryotic photosynthetic source.

Noteworthy, the two MbAPX sequences occupy distinct positions within the APX clade: while Mb18816 branches close to the land plant cytosolic and peroxisomal sequences – which are thought to be the result of a duplication event in the lineage leading to plants (Teixeira *et al.*, 2004), Mb13407 associates with plastid APXs; specifically, Mb13407 branches with *Ostreococcus* chloroplast APXs (Fig. 1a and Supporting information Fig. S1). *Ostreococcus* belongs to a paraphyletic assemblage of green algae, known as the prasinophytes, which comprise the descendants of the primitive algae from which all green algal lineages, including the ancestors of land plants, evolved. Consistent with this affiliation, the Mb13407 APX exhibits the two insertions that distinguish the plastid APX forms from the nonplastid forms (Teixeira *et al.*, 2004); furthermore, the predicted amino acid sequence of the second insertion is very similar to the prasinophyte counterparts (Fig. 1b). Likewise, as expected for nonchloroplastic APXs, Mb18816 lacks the chloroplast-specific insertions (Fig. 1b).

Interestingly, an animal-type peroxidase (IPR002007) is also present in the *M. brevicollis* genome (ID 26049),

Fig. 1 Cytochrome *c* (CCP) and ascorbate (APX) peroxidases. (a) Bayesian analysis (96 taxa, 188 sites; number at nodes are posterior probabilities) of selected CCP (shaded in yellow) and APX sequences (shaded in grey, plastid APX are shaded in green and land plant peroxisomal and cytoplasmic APXs are shaded in pink and blue respectively) from all major eukaryotic lineages for which complete sequences are available (fungi are in brown, excavates are in blue, chromalveolates are in pink, red algae are in red, green algae and land plants are in green and arrows indicate *Monosiga* sequences). Maximum likelihood analyses suggest similar relationships; bootstrap values (200 replicates) for key nodes are indicated in italics, below the posterior probability values. Numbers following species names are Uniprot IDs – if composed of both letters and numbers, or JGI IDs – if consist of only numbers. Phylogenetic analyses using additional APX sequences do not affect the observed affiliations (Supporting Information Fig. S1). (b) Partial alignment of the two *Monosiga* APX sequences (in purple) and selected nonplastid (in blue) and plastid (in green) APXs, showing the location and the predicted amino acid sequence of the two insertions characteristic of plastid APXs.



arguing that the two APX sequences were added to an existing peroxidase gene complement comprising at least a CCP and an animal peroxidase. The strong level of sequence conservation between *Monosiga* and other APXs indicate that they encode functional proteins (several ESTs have also been sequenced; http://genome.jgi-psf.org/Monbr1/). Notably, cellular localization prediction tools suggest that Mb13407 and Mb18816 are functioning in the mitochondria and cytosol respectively; the retargeting of a chloroplast protein to another organelle following a gene transfer event is not without precedent (e.g. Hannaert *et al.*, 2003).

Metacasapses

Metacaspases are cysteine proteases related to caspases (which function as essential executors of programmed cell death in animals) and procaspases (found in animals and the amoebozoan, *Dictyostelium discoideum*) (Uren *et al.*, 2000). Metacaspase sequences have been reported among protozoans, fungi, algae and plants (e.g. Bidle & Falkowski, 2004; Vercammen *et al.*, 2007) and are also thought to be involved in responses to stress and in cell death (e.g. Bidle & Falkowski, 2004). Interestingly, in contrast to protists and fungi, land plants possess two types of metacaspases, types I and II – the main difference being the presence of an insertion between the p20- and p10-like subunits in the latter (Uren *et al.*, 2000).

Our searches for metacaspases in *Monosiga* identified two such sequences in the genome of *M. brevicollis* (IDs 32666 and 29825) and one in the *M. ovata* EST database (MNL00001674). Surprisingly, one of the two *M. brevicollis* sequences, Mb29825, contains the insertion characteristic of plant type II metacaspases, which we also found in green algae (Fig. 2a) but not in any other lineages. Phylogenetic analyses support the inclusion of the two *M. brevicollis* metacaspase sequences in the type I and type II metacaspase groups; however, they also suggest an unexpected affiliation between the *M. brevicollis* type I metacaspase and their algal/plant counterparts, to the exclusion of fungal and amoebozoan metacaspase sequences (Fig. 2b). A 'plant-like' nature for the *Monosiga* type I metacaspase sequence is further supported by the presence at the N-terminus of the predicted protein of an LSD1-type Zn finger (IPR005735), which is a negative regulator of cell death in plants (Dietrich *et al.*, 1997). This specific association is only found in land plants (and probably in their close green algal ancestors) and, although both LSD1 Zn fingers and type I metacaspases are present in other protists (ciliates, trypanosomatids and green algae), they are never found together; moreover, LSD1 fingers are not known in fungi and animals, and no other LSD1-containing proteins were found in *Monosiga*.

Although highly divergent metacaspase-like sequences have been reported in bacteria (Bidle & Falkowski, 2004), they do not exhibit either the domain organization characteristic of plant type I metacaspases (in fact, LSD1 domains are not found in bacteria) or the presence of the insertion characteristic of type II metacaspases in green algae in land plants. Thus, these specific features shared exclusively by *Monosiga* and green algal and plant metacaspases, to the exclusion of all other metacaspases including bacterial counterparts, argue strongly for the *Monosiga* sequences being acquired from a photosynthetic eukaryote lineage related to the extant green algal/land plant lineage. Phylogenetic analyses including EST sequences from additional algal and glaucophyte type I metacaspases (Supporting Information Fig. S2) suggest that the *Monosiga* sequence was acquired from an ancestral algal lineage that diverged before the split between the Streptophyta (land plants and their algal ancestors) and the Chlorophyta (the rest of green algae).

The high level of sequence conservation between the two *Monosiga* metacaspases and their counterparts, as well as the fact that at least one of the two metacaspase genes is transcribed indicate that these sequences are functional. Similar to the peroxidase case discussed earlier, as type II metacaspases are not known in unikont lineages, the acquisition of this sequence can be viewed as an addition to the existing gene complement of *Monosiga*; on the other hand, if a unikont type I metacaspase was present in the choanoflagellate ancestor, the extant *Monosiga* type I sequence would be indicative of a functional replacement event.

Evolutionary implications

The inferred affiliations between the *Monosiga* and the Viridiplantae (i.e. green algae and land plants) homologues documented above are unlikely to be artefactual, as all the cases reported here are supported by more than one type of evidence, including phylogenetic distribution (summarized in Fig. 3), domain organization, specific insertions and sequence affiliations (Figs 1 and 2). Although a sister relationship between Metazoa (i.e. animals) and Viridiplantae (to the exclusion of fungi) was suggested by some analyses (Philip *et al.*, 2005; Stiller, 2007), we do not believe that our findings can be interpreted as a reflection, or in support, of this scenario. For instance, the phylogenetic distribution of APXs – which is limited to plastid-containing lineages, is inconsistent with this scenario. Thus, based on the available and under the current understanding of the 'eukaryotic tree' (Fig. 3), we argue that the four *Monosiga* sequences coding for stress-related proteins have been acquired from the green algal/land plant lineage. Notably, at least three of the four sequences reported here represent additions to the choanoflagellate gene complement.

Having argued for an algal origin for these *Monosiga* stress-related genes, when and how were they acquired? The presence of algal-related sequences in both *M. brevicollis* and *M. ovata* suggests that the acquisition events took place before the divergence of the two

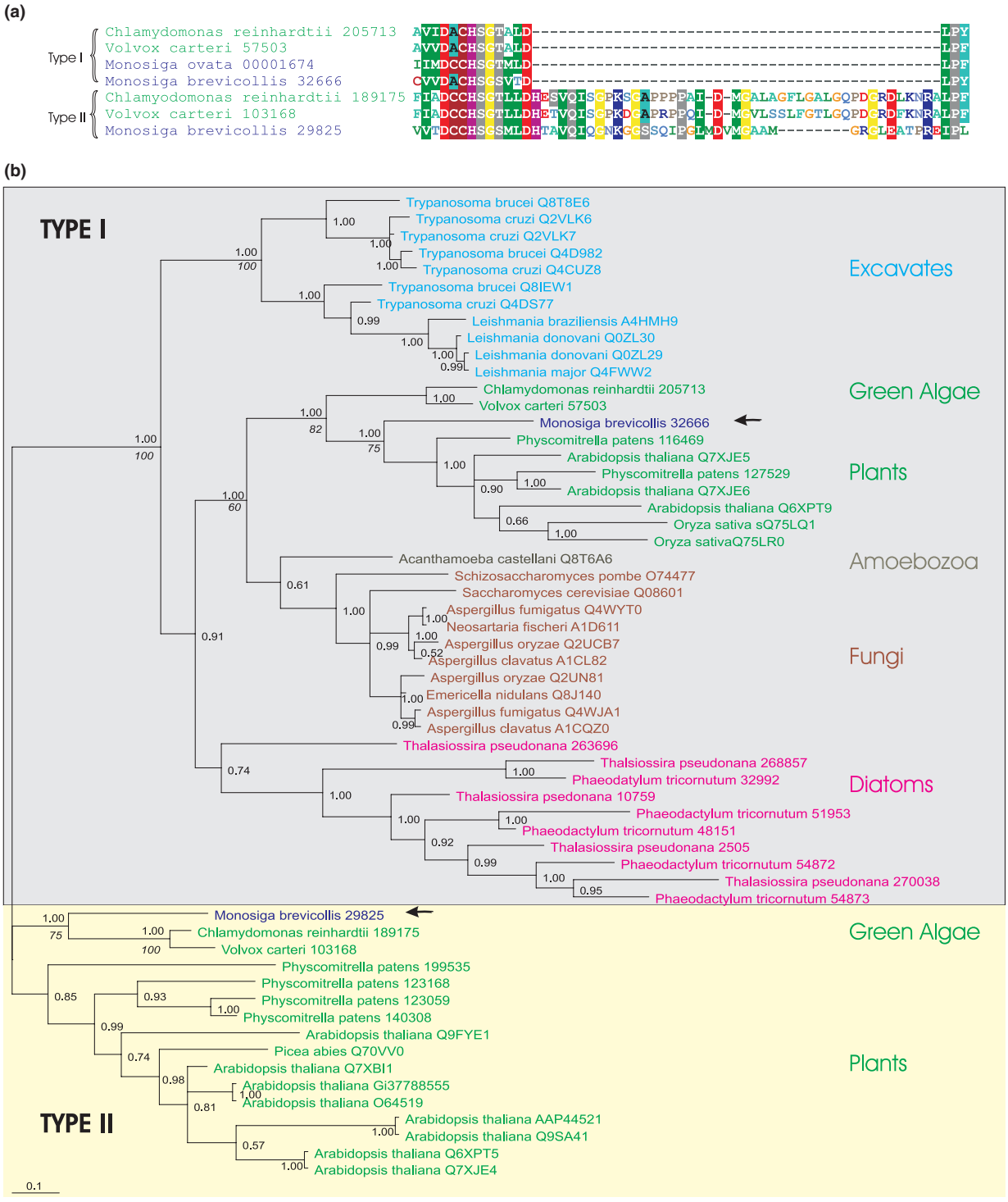


Fig. 2 Type I and type II metacaspases. (a) Partial alignment of *Monosiga* (in purple) and green algal (in green) type I and type II metacaspases showing the conserved location and predicted amino acid sequence of the insertion specific for type II metacaspases. (b) Bayesian analysis (58 taxa, 116 sites) of selected type I (grey-shaded area) and type II (yellow-shaded area) metacaspases from all major eukaryotic lineages for which complete sequences are available (the highly diverged *Ostreococcus* type I metacaspase sequences, A4S815, Q00UP8, were excluded). Maximum likelihood analyses predict similar relationships; bootstrap values (200 replicates) for key nodes are indicated in italics, below the posterior probability values.

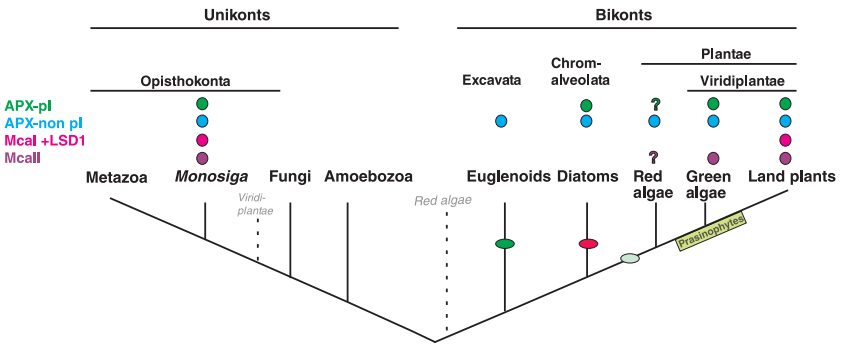


Fig. 3 Phylogenetic distribution of APXs and metacaspases. Coloured dots denote the presence, in the lineage below the dot, of the corresponding sequence indicated on the left (pl = plastid; McaI = type I metacaspase; McaII = type II metacaspase); question marks denote: (i) a putative red algal type II metacaspase based on several *Porphyra* ESTs (GenBank accessions: AU189679, AU189520, AU186857, AU188368, AU194902 and AV433034) – although no metacaspase sequences could be identified in the available red algal genomes); and (ii) the presence of hybrid APX sequences in red algae. Relative position of the main eukaryotic lineages for which genomic information is available is based on Cavalier-Smith (2003) and Stechmann & Cavalier-Smith (2003); distances between branches are not indicative of evolutionary distance or time. Alternative branching patterns proposed for red algae (Nozaki *et al.*, 2007) and/or Viridiplantae (Philip *et al.*, 2005; Stiller, 2007) are indicated with dashes. Shaded ovals indicate the primary chloroplast acquisition in the last common ancestor of Plantae (light blue-green oval), and the acquisition of secondary plastids from red (red oval) or green (green oval) algae in diatoms and euglenoids, respectively.

lineages – that is, early in the evolutionary history of choanoflagellates (Cavalier-Smith & Chao, 2003). Among unicellular eukaryotes, LGT is thought to occur mainly in phagotrophic species, via ingested food ('you are what you eat' hypothesis; Doolittle, 1998), and be facilitated by the donor and recipient living in the same environment (Andersson, 2005; Andersson *et al.*, 2006). The fact that *Monosiga* are phagotrophic and both choanoflagellates and green algae are ancestrally marine groups is consistent with LGT via food. However, the same conditions can facilitate the establishment of intracellular symbioses. Although in most cases the algal endosymbionts are reduced to, and function as, secondary or tertiary plastids, in several instances the symbionts have degenerated (e.g. in apicomplexans) or have been completely lost (e.g. in oomycetes and possibly ciliates), leaving behind a number of genes that have been transferred into the host nucleus (Archibald & Keeling, 2002; Tyler *et al.*, 2006; Reyes-Prieto *et al.*, 2008). The distinction between LGT and EGT is rather difficult in cases where the symbiont was lost (Keeling & Palmer, 2001; Archibald *et al.*, 2003; Reyes-Prieto *et al.*, 2008). However, the two processes are not mutually exclusive, as the acquisition of an endosymbiont does not preclude previous or subsequent LGT events via food (see the chlorarachniophyte, *Bigelowiella natans*, case; Archibald *et al.*, 2003). In the absence of structural remnants of the symbiont, such LGTs could obscure the evidence for the past presence of an endosymbiont. These interpretations are even more difficult for: (i) old events – which is possibly the case in *Monosiga*; and/or (ii) donor lineages that have undergone extensive radiation (such as the prasinophyte algae).

Nevertheless, regardless of the transfer pathway (EGT or LGT), the algal genes that represent additions to *Monosiga*'s gene complement were most likely retained because of their selective advantage – as opposed to being stochastic events, which can be invoked in the case of functional gene replacement (Doolittle, 1998; Stechmann *et al.*, 2006). As discussed earlier, the laterally acquired sequences could have provided the primitive choanoflagellates with additional or more efficient means to cope with stress; these novel adaptive traits could then have contributed to the diversification of the choanoflagellate lineage, especially in relation to adapting to freshwater environments and/or sessile or colonial lifestyles. If some of these events occurred before the divergence of the lineage leading to animals, it is tempting to speculate that such gene transfers could have also impacted the early evolution of animals. For instance, the ability to finely tune cellular ROS levels could have been important for the co-option of ROS as signalling molecules during the early evolution of multicellular development (Blackstone, 2003, 2008), before new animal-specific traits evolved.

The presence in the early-diverged metazoan lineage, *Hydra*, of a peroxidase-like sequence thought to have been acquired early in the evolutionary history of this lineage from a green alga unrelated to the extant algal symbionts found in some *Hydra* species (Passardi *et al.*, 2007) is consistent with this scenario. The absence of homologues of the algal genes we found in *Monosiga* in the extant animal lineages for which we have data should not be necessarily interpreted as evidence against the acquisition of these sequences before the divergence of Metazoa, as several other stress-related genes present in unikonts have been specifically lost in the animal lineage. These include the mitochondrial CCP (found in fungi and choanoflagellates, but absent from animals) and the unikont type I metacaspase (absent in metazoans – although present in

Amoebozoa and fungi). Additional genome data from early-diverged metazoan lineages should help decipher the evolutionary history of these events and address their potential contribution to the early evolution of Metazoa (noteworthy, peroxidase- and metacaspase-like sequences can be identified in the whole-genome shotgun sequences of a sponge; http://compagen.zoologie.uni-kiel.de/). It is possible that other pathways have been affected by LGT in *Monosiga*, and further studies will assess the extant and evolutionary significance of such transfers. Overall, this study documents four cases of adaptive eukaryote-to-eukaryote lateral gene transfer and argues that such transfers could have contributed to the evolution and diversification of eukaryotic lineages.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 Bayesian analysis of ascorbate peroxidases including additional sequences.

Figure S2 Bayesian analysis of type I metacaspases from *Monosiga brevicollis*, land plants, green algae and glaucophytes.

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