Conservation of Nonsense-Mediated mRNA Decay Complex Components Throughout Eukaryotic Evolution

Barry Causier1, Zhen Li2,3, Riet De Smet2,3, James P. B. Lloyd1, Yves Van de Peer2,3,4 & Brendan Davies1

Nonsense-mediated mRNA decay (NMD) is an essential eukaryotic process regulating transcript quality and abundance, and is involved in diverse processes including brain development and plant defenses. Although some of the NMD machinery is conserved between kingdoms, little is known about its evolution. Phosphorylation of the core NMD component UPF1 is critical for NMD and is regulated in mammals by the SURF complex (UPF1, SMG1 kinase, SMG8, SMG9 and eukaryotic release factors). However, since SMG1 is reportedly missing from the genomes of fungi and the plant Arabidopsis thaliana, it remains unclear how UPF1 is activated outside the metazoa. We used comparative genomics to determine the conservation of the NMD pathway across eukaryotic evolution. We show that SURF components are present in all major eukaryotic lineages, including fungi, suggesting that in addition to UPF1 and SMG1, SMG8 and SMG9 also existed in the last eukaryotic common ancestor, 1.8 billion years ago. However, despite the ancient origins of the SURF complex, we also found that SURF factors have been independently lost across the Eukarya, pointing to genetic buffering within the essential NMD pathway. We infer an ancient role for SURF in regulating UPF1, and the intriguing possibility of undiscovered NMD regulatory pathways.

Transcript decay is a key determinant of gene expression levels and the rate of change of expression. Eukaryotic cells have evolved pathways to regulate transcript abundance, both to modulate expression and for quality control. Nonsense-mediated mRNA decay (NMD) is a quality control mechanism that eliminates aberrant mRNAs containing premature translation termination codons (PTCs). NMD also influences the abundance of up to 10% of non-aberrant transcripts in animals, fungi and plants, suggesting that it is an important post-transcriptional regulator of gene expression. NMD is activated when translating ribosomes stall at termination codons in specific contexts. In mammals, the first step in NMD is formation of a transient ‘SURF’ complex on the stalled ribosome. SURF is composed of SMG1, SMG8 and SMG9 (collectively known as SMG1C), together with the core NMD factor UPF1 and eukaryotic release factors 1 and 3 (eRF1 and 3). Phosphorylation of UPF1 by the SMG1 phosphatidylinositol 3-kinase related kinase (PIKK), is central to NMD. SMG8 and SMG9 act as a scaffold for the interaction between SMG1 and UPF1, but also negatively regulate SMG1 kinase activity, maintaining UPF1 in an unphosphorylated, inactive state within the SURF complex. Interaction between UPF1 and the other core NMD factors, UPF2 and UPF3, promotes formation of an mRNA decay-initiating complex (Fig. 1A). UPF2 and UPF3 antagonise the inhibitory effects of SMG8 and SMG9, allowing SMG1 to phosphorylate and activate UPF1 (reviewed). In common with other PIK-kinases, SMG1 phosphorylates Serine (S) or Threonine (T) residues within a S/TQ dipeptide motif. NMD requires phosphorylation of the N- and C-termini of the UPF1 protein, which are enriched for S/TQ dipeptides, leading to recruitment of SMG5, SMG6 and SMG7 by phospho-UPF1 and transcript decay (Fig. 1A).

1Centre for Plant Sciences, Faculty of Biological Sciences, University of Leeds, Leeds, LS2 9JT, UK. 2Department of Plant Biotechnology and Bioinformatics, Ghent University, Technologypark 927, B-9052, Gent, Belgium. 3VIB Center for Plant Systems Biology, Technologypark 927, B-9052, Gent, Belgium. 4Department of Genetics, Genomics Research Institute, University of Pretoria, Private Bag X20, Pretoria, 0028, South Africa. Correspondence and requests for materials should be addressed to B.C. (email: b.e.causer@leeds.ac.uk).
Figure 1. NMD factors and PIKKs have been retained and lost throughout eukaryote evolution. (A) Key steps in the NMD process. (1) The transient SURF complex (SMG1 + UPF1 + eRFs) forms at a ribosome stalled at a stop codon. In the SURF complex, SMG8 and SMG9 prevent UPF1 activation by inhibiting its phosphorylation by SMG1. SMG1, SMG8 and SMG9 are also important for recruiting UPF1 into the SURF complex. (2) Following interaction between UPF2 and UPF3, the SURF complex is remodelled into a transcript decay-initiating complex. This disrupts the SMG8/SMG9-mediated inhibition of SMG1, allowing it to phosphorylate and activate UPF1, which interacts with transcript decay-inducing factors, including SMG5-7 (3). (B) A total of 312 eukaryote genomes were surveyed for the presence or absence of genes encoding core NMD factors, PIKKs and subunits of the SURF complex. Numbers to the right indicate the number of individual species within a particular eukaryotic group that were examined. The summarized phylogeny to the left shows the relationships between the major eukaryotic groups studied. The percentage of species within each group where genes encoding these factors were found is represented by heat maps, as shown to the right. Where only
UPF1 and SMG1 are multifunctional proteins with cellular roles independent of NMD. In metazoans UPF1 is a component of the Staufen-mediated mRNA decay pathway. In addition, UPF1 is an important regulator of genome integrity. Similarly, SMG1 has been implicated in DNA damage responses and identified as a tumor suppressor linked to different cancers, stimulating interest in SMG1 as a novel target for drug therapies and a biomarker for cancer prognosis. SMG1, and the related PIKKs ATM and ATR regulate genome integrity, through phosphorylation of both UPF1 and p53. However, an NMD compromised phenotype has only been observed for mutants or knockdowns of SMG1, suggesting that SMG1 is the only PIKK that phosphorylates UPF1 to promote NMD, at least in metazoans.

Our understanding of NMD is less advanced outside of animal systems, but similarities and differences are emerging. For example, although UPF1 is phosphorylated in the model fungus Saccharomyces cerevisiae, the SMG1 kinase gene is missing from its genome. SMG1 is also missing from the genome of the model plant Arabidopsis thaliana, although UPF1 is phosphorylated in plants at the N- and C-termini, which act redundantly in NMD. The apparent lack of a kinase to activate UPF1 raises unresolved questions about the mechanism of NMD in these organisms.

Until recently it was thought that SMG1, and hence the SURF complex, was only found in animals. However, we recently demonstrated that A. thaliana is atypical, since SMG1 is widespread in the plant kingdom, where it also functions in NMD. The involvement of SMG1 in NMD in both animals and plants indicates that its NMD function likely predates the divergence of eukaryotes. However, loss or depletion of SMG1 in different branches of the eukaryote domain paints a more complex picture. Depletion of SMG1 is fatal in mammals and disrupts NMD in C. elegans and the moss plant Physcomitrella patens. In contrast, evolutionary losses of SMG1 from S. cerevisiae and A. thaliana, or induced loss of SMG1 in Drosophila and zebrafish are tolerated.

Here we take a genomic approach to provide a framework to interpret the functional data from different species and to understand the origins, mechanism and evolution of NMD. By mapping the distribution of SURF complex components (UPF1, SMG1, SMG8 and SMG9), other core NMD factors (UPF2 and UPF3) and PIKKs (ATM and ATR) across a comprehensive selection of 312 genomes spanning eukaryotic evolution we provide evidence that all known SURF complex components were present in the last eukaryotic common ancestor (LECA). However, our discovery of multiple independent losses of the SURF complex during eukaryotic evolution indicates the existence of undiscovered NMD regulatory processes, which might also be of ancient origin. Our findings have implications for the fields of gene regulation and RNA processing.

Results

By screening 312 eukaryotic genomes we determined the current distribution of the genes encoding the components of the NMD-promoting SURF complex (SMG1, SMG8, SMG9, UPF1), other core NMD factors (UPF2, UPF3) and other PIKK kinases that target UPF1 (ATM, ATR). The complete dataset is presented in Tables S1–S3.

Components of the NMD pathway were present in the earliest eukaryotes. A recent global phylogeny classified eukaryotes into three megagroups: the Excavata, the Amorphea (which includes animals, fungi and Amoebozoa), and the Diaphoretickes (plants, Stramenopiles, Alveolata and Rhizaria) (see Fig. 1B). As an early branching sister group to the eukaryotes, the Excavata have been the focus of considerable study to understand eukaryotic origins. Since NMD has been shown to be at least partially active in members of the Excavata group, we searched the genomes of seven Excavata species for genes encoding NMD and SURF factors (Fig. 1B and Table S1). Although UPF1 is conserved in all seven species, components of the SMG1C complex (SMG1, SMG8 and SMG9) that regulates UPF1 activity for NMD in mammals are restricted to Naegleria gruberi, which has both SMG1 and SMG9. N. gruberi is a free-living organism, whereas the other Excavates used in this study are obligate parasites, which are often characterised by highly reduced genomes (reviewed).

To our knowledge, this is the first report of SMG1C components in these deep diverging lineages of eukaryotes. A previous study reported the identification of an SMG1 homologue in Giardia lamblia (Genbank accession no. AY095369), but BLAST homology searches and protein domain analyses clearly show this to be more closely related to a different PIKK (mTOR) than to SMG1.

In animals UPF1 phosphorylation occurs when the core NMD factors UPF2 and UPF3 interact with the SURF complex to form an mRNA decay-initiating complex (Fig. 1A). UPF1 and UPF2 have previously been reported in yeast, animals, plants and the Excavata and the Diaphoretickes (plants, Stramenopiles, Alveolata and Rhizaria) (see Fig. 1B). As an early branching sister group to the eukaryotes, the Excavata have been the focus of considerable study to understand eukaryotic origins. Since NMD has been shown to be at least partially active in members of the Excavata group, we searched the genomes of seven Excavata species for genes encoding NMD and SURF factors (Fig. 1B and Table S1). Although UPF1 is conserved in all seven species, components of the SMG1C complex (SMG1, SMG8 and SMG9) that regulates UPF1 activity for NMD in mammals are restricted to Naegleria gruberi, which has both SMG1 and SMG9. N. gruberi is a free-living organism, whereas the other Excavates used in this study are obligate parasites, which are often characterised by highly reduced genomes (reviewed).

To our knowledge, this is the first report of SMG1C components in these deep diverging lineages of eukaryotes. A previous study reported the identification of an SMG1 homologue in Giardia lamblia (Genbank accession no. AY095369), but BLAST homology searches and protein domain analyses clearly show this to be more closely related to a different PIKK (mTOR) than to SMG1.

In animals UPF1 phosphorylation occurs when the core NMD factors UPF2 and UPF3 interact with the SURF complex to form an mRNA decay-initiating complex (Fig. 1A). UPF1 and UPF2 have previously been reported in yeast, animals, plants and the Excavata and the Diaphoretickes (plants, Stramenopiles, Alveolata and Rhizaria) (see Fig. 1B). As an early branching sister group to the eukaryotes, the Excavata have been the focus of considerable study to understand eukaryotic origins. Since NMD has been shown to be at least partially active in members of the Excavata group, we searched the genomes of seven Excavata species for genes encoding NMD and SURF factors (Fig. 1B and Table S1). Although UPF1 is conserved in all seven species, components of the SMG1C complex (SMG1, SMG8 and SMG9) that regulates UPF1 activity for NMD in mammals are restricted to Naegleria gruberi, which has both SMG1 and SMG9. N. gruberi is a free-living organism, whereas the other Excavates used in this study are obligate parasites, which are often characterised by highly reduced genomes (reviewed).

To our knowledge, this is the first report of SMG1C components in these deep diverging lineages of eukaryotes. A previous study reported the identification of an SMG1 homologue in Giardia lamblia (Genbank accession no. AY095369), but BLAST homology searches and protein domain analyses clearly show this to be more closely related to a different PIKK (mTOR) than to SMG1.

In animals UPF1 phosphorylation occurs when the core NMD factors UPF2 and UPF3 interact with the SURF complex to form an mRNA decay-initiating complex (Fig. 1A). UPF1 and UPF2 have previously been reported in yeast, animals, plants and the Excavata and the Diaphoretickes (plants, Stramenopiles, Alveolata and Rhizaria) (see Fig. 1B). As an early branching sister group to the eukaryotes, the Excavata have been the focus of considerable study to understand eukaryotic origins. Since NMD has been shown to be at least partially active in members of the Excavata group, we searched the genomes of seven Excavata species for genes encoding NMD and SURF factors (Fig. 1B and Table S1). Although UPF1 is conserved in all seven species, components of the SMG1C complex (SMG1, SMG8 and SMG9) that regulates UPF1 activity for NMD in mammals are restricted to Naegleria gruberi, which has both SMG1 and SMG9. N. gruberi is a free-living organism, whereas the other Excavates used in this study are obligate parasites, which are often characterised by highly reduced genomes (reviewed).

To our knowledge, this is the first report of SMG1C components in these deep diverging lineages of eukaryotes. A previous study reported the identification of an SMG1 homologue in Giardia lamblia (Genbank accession no. AY095369), but BLAST homology searches and protein domain analyses clearly show this to be more closely related to a different PIKK (mTOR) than to SMG1.

In animals UPF1 phosphorylation occurs when the core NMD factors UPF2 and UPF3 interact with the SURF complex to form an mRNA decay-initiating complex (Fig. 1A). UPF1 and UPF2 have previously been reported in yeast, animals, plants and the Excavata and the Diaphoretickes (plants, Stramenopiles, Alveolata and Rhizaria) (see Fig. 1B). As an early branching sister group to the eukaryotes, the Excavata have been the focus of considerable study to understand eukaryotic origins. Since NMD has been shown to be at least partially active in members of the Excavata group, we searched the genomes of seven Excavata species for genes encoding NMD and SURF factors (Fig. 1B and Table S1). Although UPF1 is conserved in all seven species, components of the SMG1C complex (SMG1, SMG8 and SMG9) that regulates UPF1 activity for NMD in mammals are restricted to Naegleria gruberi, which has both SMG1 and SMG9. N. gruberi is a free-living organism, whereas the other Excavates used in this study are obligate parasites, which are often characterised by highly reduced genomes (reviewed).

To our knowledge, this is the first report of SMG1C components in these deep diverging lineages of eukaryotes. A previous study reported the identification of an SMG1 homologue in Giardia lamblia (Genbank accession no. AY095369), but BLAST homology searches and protein domain analyses clearly show this to be more closely related to a different PIKK (mTOR) than to SMG1.
PIKK regulators of UPF1 activity are ancient protein kinases. The phosphorylation of UPF1 by PIKK kinases is crucial for its varied activities. While SMG1 is the only PIKK to activate UPF1 for both NMD, SMG1 and the related PIKKs ATM and ATR all phosphorylate UPF1 upon DNA damage. We find SMG1, ATM and ATR genes in the genomes of species from both the Amorphea and the Diaphoretickes (Fig. 1B, Tables S1 and S3). In contrast to a previous report, the *N. gruberi* genome contains genes encoding SMG1, ATM and ATR (Table S3). Other studies have identified ATM and/or ATR in the parasitic Excavate species *Trichomonas*, *Leishmania* and *Trypanosoma*, but not *Giardia*.

Consistent with this, we found ATM and ATR genes in the genomes of *Trichomonas vaginalis*, *T. brucei* and *Leishmania major*, but not in *Giardia intestinalis*, *G. lamblia* or *Spironucleus salomonicensis* (Table S3). These findings indicate an ancient origin for PIK kinases, although we also observe rare, independent PIKK losses throughout eukaryote evolution. Of the eight Alveolata genomes examined, we failed to identify ATM or ATR in six. The Alveolata can be separated into three distinct groups: ciliates, dinoflagellates and apicomplexans. Loss of ATM and ATR from both *Paramecium tetraurelia* and *Tetrahymena thermophila* suggests that these PIKKs were lost at the base of the ciliates (Table S3). No dinoflagellate genomes were examined here, but both ATM and ATR were found within the apicomplexans (*Plasmodium vivax* and *Toxoplasma gondii*), although ATR was missing from all 5 *Plasmodium* species examined, while ATM was only found in *P. vivax*. Together, this suggests multiple losses of these kinases during Alveolata evolution. SMG1 is also missing from all Alveolata genomes examined (see below), which suggests that these largely parasitic eukaryotes can survive without UPF1-activating PIKKs. We were also unable to find ATM in the genomes of certain fungal groups, including the Glomeromycte and its sister group the Mucoromycyte, suggesting loss of ATM from the common ancestor of these fungi. ATM is also missing from the majority of Microsporidia genomes examined (Table S3), suggesting that it has been lost independently throughout fungal evolution.

ATM, ATR and SMG1 are found throughout the Eukarya domain, yet despite their ancient origins our findings suggest that some eukaryotes can tolerate the loss of all UPF1-activating PIKKs, implying that there are alternative mechanisms to regulate UPF1 activity.

SMG1 has been independently lost from diverse eukaryotic lineages. SMG1, the only kinase known to activate UPF1 for NMD, is essential for mammalian viability, but its role in other species is less clear. Phylogenetic profiling revealed that SMG1 is missing from 124 of the 312 genomes surveyed (Fig. 1C and Table S1). Many of the observed SMG1 losses are from fungi (SMG1 is in just 24 of the 94 fungal genomes examined, Table S1). SMG1 is absent from Ascomycota and Basidiomycota fungal genomes (collectively known as the Dikarya; Fig. 2). Phylogenetic analyses place the Glomeromycte (the arbuscular mycorrhizae) and Mucoromycota as sister groups to the Dikarya (see Fig. 2). Despite the close position of these fungal groups to the Dikarya, from which SMG1 is absent, SMG1 is retained in genomes of both Glomeromycte and Mucoromycota (Fig. 2 and Table S1). Furthermore, we also found SMG1 in genomes of a number of basal fungal groups (Fig. 2). Therefore, in contrast to current opinion, SMG1 is present in the genomes of several fungal clades although multiple independent losses of SMG1 have occurred throughout fungal evolution.

The fungal and animal kingdoms both belong to the Amorphea megagroup of eukaryotes (Fig. 1B). Nevertheless, in contrast to the high frequency of loss of SMG1 from fungal genomes, loss of SMG1 from animal
genomes is extremely rare (Fig. 1B). Of the 121 animal genomes examined, we failed to find SMG1-like genes in only three instances: Mnemiopsis leidyi (Eumetazoa), Trichinella spiralis (Nematoda) and Megaselia scalaris (Arthropoda) (Table S1). The Diaphoretickes megagroup of eukaryotes includes plants, Stramenopiles, Alveolata and Rhizaria (see Fig. 1B). SMG1 is absent from 12 of the 19 Stramenopile genomes examined and all of the Alveolata genomes (Table S1). In contrast, SMG1 is present in the vast majority of green plant genomes, including relatives of the earliest aquatic plants (the Chlorophyta), the earliest diverging land plants (e.g. the moss Physcomitrella patens), the earliest diverging vascular plants (e.g. the lycophyte Selaginella moellendorffii), and flowering plants (Fig. 3A and Table S1). Loss of SMG1 was only seen in the Chlorophyta (60% of the examined genomes lacked SMG1) and a single group of land plants, the Brassicaceae, to which the model plant Arabidopsis thaliana belongs (Fig. 3B). As previously reported, SMG1 is missing from the genome of A. thaliana yet is present in the genome of its nearest relative Arabidopsis lyrata. Since these species diverged only 5–10 mya this represents a recent independent loss (Fig. 3B).

In summary, while UPF1 has been maintained in almost all eukaryotic genomes, SMG1 has been independently lost in many lineages. Despite its absence from the genome of the model species S. cerevisiae and most fungal groups, SMG1 has been retained in at least four major fungal lineages. In contrast, losses of SMG1 from animal and land plant genomes are rare.

**SMG1C components are co-eliminated.** The SMG1C complex includes SMG1 and the factors that control it in mammalian NMD. Phylogenetic profiling studies suggest that functionally linked genes are often gained and lost together in evolution. To examine whether components of the SMG1C have been retained or lost in concert across eukaryotic evolution, we surveyed their phylogenetic distribution. SMG1, SMG8 and SMG9 are missing from 124, 167 and 157 of the 312 genomes examined, respectively, with loss of all three genes being found in 100 cases (Fig. 1C). Underlying this is a small number of SMG1C losses at the base of major clades (Fig. 1D). For example, all three SMG1C genes are missing from the Ascomycota and Basidiomycota fungal genomes, suggesting that the entire SMG1C was lost from the common ancestor of the Dikarya. The full complement of
SMG1C genes was also likely lost from the common ancestor of Microsporidia. Beyond the fungi, patterns of gene loss suggest that the entire SMG1C was also lost at the base of the Alveolata (Fig. 1D). In addition to the loss of SMG1C at the base of major clades, many independent losses of the entire SMG1C can be seen within different lineages. For example, within the Chlorophyta all SMG1C genes are missing from all species of Ostreococcus examined (Fig. 1D).

There are a limited number of examples of more recent co-elimination of genes encoding SMG1C components. For example, the plant A. thaliana split from its nearest relative A. lyrata just 5–10 mya 55 yet A. thaliana has lost all of the SMG1C genes, whilst they have all been retained in A. lyrata (Fig. 3B and Table S1). Genome synteny reveals that the expected locations of SMG1, SMG8 and SMG9 map to distinct regions of the A. thaliana genome (Figure S1), showing that each gene was lost independently. Fragments of SMG1 and SMG9 are detectable in the A. thaliana genome (Figure S1), confirming these recent gene losses. Examination of patterns of SMG1C gene loss in land plants suggests a complex history within the Brassicaceae, which includes Arabidopsis. As mentioned above, SMG1C is missing from A. thaliana but is retained in A. lyrata. All three SMG1C components appear to be lost in Capsella, which is sister to Arabidopsis (Fig. 3B), with a fragment of SMG1 remaining that is different to that found in the A. thaliana genome (Figure S1a). Therefore, SMG1C has been independently lost at least twice in the Brassicaceae. Loss of individual SMG1C components is commonplace amongst the Brassicas. The extraordinary propensity of Brassicaceae to independently lose genes encoding SMG1C components, which contrasts starkly with their retention in other plant groups, could be explained by an alternative mode of NMD regulation in this plant group.

Assembly of the SMG1C depends on a domain of SMG1 known as the C-insertion, which is not found in other PIKKs 58. In mammals, the C-insertion is bound by SMG8 and SMG9 and recruits and regulates UPF1 activity and NMD. The retention of SMG1C throughout eukaryote evolution suggests that this regulatory mechanism is also conserved. However, the C. elegans SMG1 protein lacks the C-insertion and SMG8 is reportedly not required for nematode NMD 58,59. To determine whether this may be the case in other eukaryotes, we examined SMG1 protein sequences for the presence of a C-insertion. We found that SMG1 proteins of all Diaphoretickes examined contain the C-insertion, as does the SMG1 protein from the Excavate N. gruberi (Fig. 4). In contrast, however, we identified multiple examples in different Amorphea lineages where the C-insertion is missing from SMG1 (Fig. 4). Although the C-insertion recruits SMG8 and SMG9, loss of this region from the SMG1 protein does not correlate with loss of SMG8 or SMG9. Taken together, the data suggest that the ancestral SMG1 protein contained the C-insertion and that it may have had the potential to recruit SMG8 and SMG9 into a regulatory SURF complex early in eukaryotic evolution. Furthermore, our findings suggest that in species where the C-insertion has been lost, alternative SURF-independent mechanisms of UPF1-regulation or alternative ways to recruit SMG8 and SMG9 could have evolved.

In summary, we find multiple independent instances of loss of the entire SMG1C, consistent with a mutually dependent role in NMD. The high frequency of losses of SMG1C components suggests that in many species, undiscovered SURF-independent mechanisms may exist to regulate the function of UPF1 in NMD, and that these too may be ancient in origin. Interestingly however, we did not find a single instance of complete loss of the SMG1C within the animal kingdom. It appears that different kingdoms, groups and species exhibit different capacities to tolerate the loss of SMG1C, possibly reflecting the efficiency of any alternative systems to regulate UPF1.

Ancient loss of SMG1 correlates with co-loss of terminal UPF1 S/TQ dipeptide motifs. In mammalian NMD, SMG1 catalyses the phosphorylation of UPF1 at Serine or Threonine residues within clusters of S/TQ dipeptides at its N- and C-termini (Fig. 5). To understand how the multiple independent loss of SMG1 could alter the way UPF1 is regulated, we compared the S/TQ dipeptide distribution in UPF1 in species where SMG1 was lost early or late in evolution. SMG1 was lost at the base of the fungal Dikarya group, over 580 mya, making this an ancient loss. Unlike human UPF1, Dikarya UPF1 proteins are relatively S/TQ poor at the N- and C-termini (Fig. 5 and Table S5). In contrast, UPF1 proteins from fungal lineages in which SMG1 has been retained contain extensive clusters of the S/TQ dipeptides at their N- and C-termini (Fig. 5). Similarly, the termini of UPF1 from D. discoideum, which retains SMG1, contain numerous S/TQ dipeptides, whereas loss of SMG1 in E. histolytica, which split from Dictyostelium over 1 billion years ago, correlates with the UPF1 termini being relatively S/TQ-poor (Fig. 5). Loss of SMG1 from the early branching Excavata also correlates with reduced S/TQ frequency in the associated UPF1 proteins.

In species where SMG1 loss was more recent, extensive co-loss of S/TQ peptides in UPF1 would not be expected. SMG1 was lost from the Arabidopsis thaliana genome just 5–10 mya and its UPF1 termini remain S/TQ rich (Fig. 5 and Table S5). Indeed, A. thaliana UPF1 shares an almost identical S/TQ distribution across the entire protein with that of Arabidopsis lyrata, which has retained SMG1 (Fig. 5). Similarly, the UPF1 protein of Capsella rubella, which split from Arabidopsis approximately 14 mya is also S/TQ-rich, yet SMG1 is missing (Fig. 5). The evolutionary analysis of S/TQ dipeptide distribution and SMG1 kinase conservation across eukaryote genomes leads to two predictions. Firstly, SMG1 is likely to phosphorylate N- and C-terminal clusters of S/TQ dipeptides in eukaryotic species beyond the animals. Secondly, the correlation between early evolutionary loss of SMG1 and a corresponding loss of terminal S/TQ clusters in UPF1 indicates that any alternative, or parallel UPF1 regulatory mechanism will not involve a PIK kinase acting in the same way as SMG1.

UPF1 is also phosphorylated by the PIKKs ATM and ATR, as a response to DNA damage. Like SMG1, ATM and ATR show specificity for S/TQ dipeptides. Since ATM and ATR do not appear to play a role in NMD, it is likely that the UPF1 phosphorylation events catalyzed by these PIK kinases differ from those catalyzed by SMG1. Thus, we might expect S/TQ dipeptides not targeted by SMG1 to be maintained in species that lack SMG1, but retain ATM/ATR. Consistent with this, the UPF1 N- and C-terminal S/TQs are preferentially lost in species lacking SMG1, while those distributed throughout the remainder of the protein are retained (Table S5). In particular,
there are three clusters of S/TQ sites: one positioned at the C-terminal end of the RNA helicase domain, a second in and around the DNA helicase region, and a third in the proximity of the AAA domain of UPF1 in most species examined. These sites appear to be conserved from the earliest eukaryotes (Fig. 5 and Table S5).

Discussion

The mammalian SURF complex regulates the activation of UPF1 by phosphorylation, which is a critical step in the essential NMD pathway. However, outside of mammals the role of the SURF complex in NMD is poorly understood. Until recently, SMG1 was thought to be an animal-specific NMD factor36–38, although even amongst animals its importance for NMD remains unresolved 39–41. Recent data from plants revealed that not only does SMG1 exist beyond the animal kingdom it is also required for NMD 38. Since SMG1 is found in all three of the eukaryote megagroups, it suggests that SMG1 was present in the LECA. Furthermore, the fact that SMG1 has a role in NMD in both animals and plants, which diverged over 1 billion years ago61,62, strongly supports the idea that one of the ancestral roles of SMG1 was regulation of NMD.

Since protein complexes tend to form early in evolution and evolve as coherent units63, the presence of SMG1 in the LECA prompted us to examine whether other components of the SURF complex were also present early in the evolution of eukaryotes. Others have previously shown that the SURF components eRF1 and eRF3 are conserved throughout eukaryotes64,65, so here we focused on UPF1, SMG1, SMG8 and SMG9. Phylogenetic profiling revealed that the SURF complex components are conserved in all major eukaryote groups (Fig. 1B), suggesting that the SURF complex was present in the LECA (Fig. 6). Despite this, the data presented here and elsewhere11,38, shows that SURF components have been independently lost multiple times throughout eukaryote evolution. It is well known that SMG1 is absent from the S. cerevisiae genome and a previous analysis indicated that SMG1 had been lost early in the fungal lineage, after divergence from the animals 38. However, our analysis of 94 genomes spanning the fungal phylogeny reveals that major fungal groups, including the arbuscular mycorrhizae (Glomeromycota) and the pathogenic Mucoromycota, have retained SMG1 (Fig. 2). Additionally, we show that SMG1, SMG8 and SMG9 were co-lost at the base of the Dikarya. In contrast to the fungi, loss of SMG1C components is rare in the animal kingdom, suggesting that while fungi can compensate for the loss of this NMD regulatory complex, this may not be the case for animals. Indeed, in mammals induced loss of SMG1 is fatal39 and knockdown of SMG1, SMG8 or SMG9 impacts the health of the organism and is linked to pathological conditions in humans (reviewed12). Within the Diaphoretickes megagroup, we found multiple examples of SMG1 loss from the Stramenopiles, the Alveolata, and from plants. Loss of SMG1 from these groups, as with the fungi, correlates with loss of SMG8 and SMG9. A common feature of genome evolution is that functionally linked genes are lost together56,57. Multiple independent, co-ordinate losses of SMG1C-encoding genes throughout eukaryote evolution (Fig. 1C), supports the hypothesis that SMG1, SMG8 and SMG9 have been functionally linked since the LECA.

Figure 4. The domain structure of SMG1 proteins from the Amorphea and Diaphoretickes. The SMG1 kinase is characterised by three domains, the FAT domain (orange), the kinase domain (red) and the FATC, located at the C-terminus of the peptide (green). In addition, SMG1 contains a large C-insertion of approximately 1000 amino acids (yellow), situated between the kinase domain and the FATC. The structure of SMG1 is shown for the Excavata (N. gruberi), the Amorphea and the Diaphoretickes. Note that in the Diaphoretickes the C-insertion is consistently present, while in the Amorphea it is missing from SMG1 in several species.
Several important questions are raised by these findings. First, if the SURF complex was required for NMD in the LECA, how is NMD regulated in extant species lacking components of SURF proteins? For example, S. cerevisiae is used extensively as a model to study NMD, as is the plant A. thaliana. In both cases phosphorylation of UPF1 is required for normal NMD. Phylogenetic reconstructions of ancestral gene content suggest that ancient eukaryotic genomes were rich in genes and protein domains, and that descendant genomes have been shaped by pervasive differential gene loss. However, evolution of the genome through reductive processes has, until recently, received scant attention. It is now emerging that genome reduction is a major mode of evolution, allowing for adaptive genome streamlining that eliminates non-essential genes and other redundant genetic material. In the case of the NMD pathway, preservation of NMD regulation in species such as S. cerevisiae and A. thaliana is most likely the consequence of genetic buffering, where alternative functionally convergent pathways compensate for the loss of genes encoding the SURF complex. This would suggest that where SMG1 is missing, an alternative kinase, which may be an unrelated kinase, phosphorylates UPF1 for NMD. Throughout evolution either kinase may have been independently retained or lost, leading to the picture we see today where loss of SMG1 is tolerated due to genetic buffering (e.g. S. cerevisiae and A. thaliana), or is fatal (mammals), presumably because no fully redundant alternative kinase gene is present in these genomes. The level of recurrent gene loss (recurring patterns of lineage specific gene loss) and the impact this has on genome evolution is not yet fully appreciated. The lineage specific loss of genes such as SMG1, SMG8 and SMG9 across the Eukarya may add to the growing body of evidence for this evolutionary process. Second, where SMG1 is absent, which kinase(s) activate UPF1 for NMD? Phosphorylation of UPF1 by the PIKKs SMG1, ATM and ATR is stimulated in response to DNA damage. ATM and ATR are conserved in all eukaryotic groups, but in organisms lacking SMG1 there is no evidence that either ATM or ATR activate UPF1 for NMD. In support of this we note that ancient loss of SMG1 is correlated with the co-loss of S/TQ.
dipeptide motifs at the N- and C-terminal ends of the UPF1 protein in these species (Fig. 5 and Table S5), suggesting that in these cases regulation of UPF1 activity occurs independently of the S/TQ motif. Recent mapping of the phosphorylation sites of *S. cerevisiae* UPF1 identified Tyrosine residues important for NMD, which are conserved across eukaryotes, including one site conserved in the Excavate *N. gruberi*. It will be interesting to learn the identity of this new Tyrosine kinase, to determine whether it is evolutionarily conserved, and to discover whether it has a role in NMD across the eukaryotes.

In mammals, interaction between the SURF components SMG8-SMG9 and SMG1 requires the large C-insertion region of SMG1. The C-insertion, bound by SMG8 and SMG9, fulfills two roles: it acts as a platform for the recruitment of UPF1 into the SURF complex, and blocks UPF1 access to the active site of SMG1, regulating UPF1 activity and therefore NMD. Here we show that the ancestral SMG1 protein contained the C-insertion, suggesting that SMG1 was able to recruit SMG8 and SMG9 into a regulatory SURF complex early in eukaryote evolution. Consistent with this, the SMG1 protein of the Excavate *N. gruberi* contains the C-insertion, as do all SMG1 proteins examined from the Diaphoretickes (Fig. 4). In contrast, there are multiple examples within Amorphea lineages where the C-insertion has been lost (Fig. 4). Deletion of the SMG1 C-insertion leads to hyper-activation of the kinase, indicating that the C-insertion allows fine-tuning of SMG1 activity. It remains to be seen whether SMG1 lacking the C-insertion can recruit SMG8 and SMG9 to stabilise the UPF1-SMG1 interaction and regulate UPF1 phosphorylation. The finding that *C. elegans* SMG8 is not required for NMD suggests that this may not be the case and that protein complexes unrelated to SURF may regulate UPF1 activity in such species. It is tempting to speculate that a SURF-independent pathway of UPF1 and NMD regulation might have co-existed with SMG1 in the LECA and might still be present in some modern eukaryotes (Fig. 6).

We show that the components of the SURF complex, which regulates a key step in mammalian NMD, are widely conserved throughout eukaryotes, indicating an ancient origin. Although SMG1 is an effector of NMD in both animals and plants, it is unknown whether regulation of SMG1 activity is also conserved or how UPF1 is modified in species lacking SMG1. Our data provides a framework for future work that will establish how NMD is regulated amongst the different branches of the Eukarya.

**Materials and Methods**

**Phylogenetic profiling.** We downloaded proteins of 70 vertebrate genomes from Ensembl (release 77), 52 fungal genomes from Ensembl Fungi (release 24), 55 metazoan genomes from Ensembl Metazoa (release 24), and 37 protista genomes from Ensembl Protists (release 24). For green plants and algae, we used the 51 species available in PLAZA 3.0 and pico PLAZA. The proteins for the basal fungal lineages (i.e. in total 42 species from...
Cryptomycota, Microsporidia, Neocallimastigomycota, Chytridiomycota, Blastocladiomycota, Kickxellomycotina, Entomophthoromycotina, Mucoromycotina, and Glomeromycota) were retrieved from MycoCosm \(^7\).

We used BLASTP (2.2.30) \(^7\) to search for proteins from each of the species above that show sequence similarities to isoforms of nine human genes: SMG1 (Ensembl accession no. ENSP00000438748), SMG9 (Ensembl accession no. ENSP00000270066), UPF1 (Ensembl accession no. ENSP00000470142), UPF2 (Ensembl accession no. ENSP00000348708), UPF3A (Ensembl accession no. ENSP00000364448), UPF3B (Ensembl accession no. ENSP00000276201), ATM (Ensembl accession no. ENSP00000388058), and ATR (Ensembl accession no. ENSP00000343741). As an E-value cut-off for this BLAST-search \(1 \times 10^{-3}\) was used. For the best hits in each species a BLAST search against the human genome (E-value < 1) was performed to ensure that the hit corresponds to the query-gene as reciprocal best hit (RBH). Based on the outcome of this reciprocal blast analysis we constructed a phylogenetic profile matrix (Tables S1–S3). In this matrix, rows represent the target genomes and the columns the query-genes, whereas the values represent the bit scores of the RBH. Because gene length leads to a bias of higher bit scores for longer alignments, we normalized bit scores by the bit score of the query-genes themselves, i.e. the bit score corresponding to a one-directional hit was found we included the best hit gene in the human genome that corresponds to the hit. We performed the same analysis with eight genes in *Arabidopsis lyrata* by using SMG1 (Phytozome 12 accession no. AL3G35680), SMG8 (Phytozome 12 accession no. AL4G11880), SMG9 (Phytozome 12 accession no. AL6G16810), UPF1 (Phytozome 12 accession no. AL8G01130), UPF2 (Phytozome 12 accession no. AL4G25670), UPF3 (Phytozome 12 accession no. AL1G35280), ATM (Phytozome 12 accession no. AL5G17000), and ATR (Phytozome 12 accession no. AL7G37010) as query-genes (Tables S1–S3).

Additional BLASTP searches were performed at [https://blast.ncbi.nlm.nih.gov/Blast.cgi](https://blast.ncbi.nlm.nih.gov/Blast.cgi) for NMD-related genes in the plants Selaginella, *Brassica napus*, *Eutrema*, *Tarenaya*, the Rhodophyte *Galdieria*, the Oomycete *Aphanomyces* and the Excavates *T. vaginalis*, *S. salmonicida* and *G. intestinalis*. NMD genes were also identified in the Excavate *N. gruberi* using BLAST at [http://genome.igi-psf.org/pages/blast-query.jsf?db = Naegr1](http://genome.igi-psf.org/pages/blast-query.jsf?db = Naegr1), and in plant *B. oleraceae* using BLAST at [http://brassicadb.org/brad/blastPage.php](http://brassicadb.org/brad/blastPage.php) (Table S1).

**Genome synteny analysis.** Synteny between the available plant genome sequences was detected essentially as previously described \(^8\). We used the Plant Genome Duplication Database ([PGDD](http://chibba.agtec.uga.edu/duplication/index/home)) \(^8\) to search for collinear genomic fragments between species based on *Arabidopsis lyrata* loci: SMG1 (PGDD accession no. 16047708), SMG8 (PGDD accession no. 16039886) and SMG9 (PGDD accession no. 16037990). Fragments of SMG1 and SMG9 in the *Arabidopsis thaliana* genome, and SMG1 in the *Capsella rubella* genome were detected by taking genomic sequence between flanking genes (based on synteny; see Figure S1) and using BLASTX against *A. lyrata* ([https://blast.ncbi.nlm.nih.gov/Blast.cgi](https://blast.ncbi.nlm.nih.gov/Blast.cgi)) to identify any sequences with homology to the NMD factors.

**Protein domain analysis.** The conserved protein domains present in UPF1 sequences from the species listed in Table S5 were identified using NCBI Conserved Domain Search ([https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi](https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi)). Similarly, the positions of the SMG1 protein domains, and the presence/absence of the C-insertion were identified using the same program for the species shown in Fig. 4.

**Identification of UPF1 S/TQ dipeptides.** S/TQ sites were mapped for the UPF1 peptide sequences from the species listed in Table S5, using NetWorkIN ([http://networkin.info](http://networkin.info)) \(^9\).

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

**References**

1. Chiba, Y. & Green, P. J. mRNA degradation machinery in plants. *J. Plant Biol.* **52**, 114–124 (2009).
2. Ghosh, S. & Jacobson, A. RNA decay modulates gene expression and controls its fidelity. *Wiley Interdiscip. Rev. RNA.* **1**, 351–361 (2010).
3. He, F. et al. Genome-wide analysis of mRNAs regulated by the nonsense-mediated and 5′ to 3′ mRNA decay pathways in yeast. *Mol. Cell.* **12**, 1439–1452 (2003).
4. Mendell, J. T., Sharifi, N. A., Meyers, J. L., Martinez-Murillo, F. & Dietz, H. C. Nonsense surveillance regulates expression of diverse classes of mammalian transcripts and muted genomic noise. *Nat. Genet.* **36**, 1073–1078 (2004).
5. Rehwinkel, J., Letunic, I., Raes, J., Bork, P. & Izaurralde, E. Nonsense-mediated mRNA decay factors act in concert to regulate common mRNA targets. *RNA* **11**, 1530–1544 (2005).
6. Guan, Q. et al. Impact of nonsense-mediated mRNA decay on the global expression profile of budding yeast. *PLoS Genet.* **2**, e203 (2006).
7. Ramani, A. K. et al. High resolution transcriptome maps for wild-type and nonsense-mediated decay-defective *Caenorhabditis elegans*. *Genome Biol.* **10**, R101 (2009).
8. Rayson, S. et al. A role for nonsense-mediated mRNA decay in plants: pathogen responses are induced in Arabidopsis thaliana NMD mutants. *PLoS One* **7**, e31917 (2012).
9. Nicholson, P. et al. Nonsense-mediated mRNA decay in human cells: mechanistic insights, functions beyond quality control and the double-life of NMD factors. *Cell. Mol. Life Sci.* **67**, 677–700 (2010).
10. Schweingruber, C., Radener, S. C., Zünd, D., Yamashita, A. & Mühlemann, O. Nonsense-mediated mRNA decay - mechanisms of substrate mRNA recognition and degradation in mammalian cells. *Biochem. Biophys. Acta.* **1829**, 612–623 (2013).
11. Yamashita, A. Role of SMG-1-mediated Upf1 phosphorylation in mammalian nonsense-mediated mRNA decay. *Genes Cells* **18**, 161–175 (2013).
12. Fatscher, T., Boehm, V. & Gehring, N. H. Mechanism, factors, and physiological role of nonsense-mediated mRNA decay. *Cell. Mol. Life Sci.* **72**, 4523–4544 (2015).
59. Rosains, J. & Mango, S. E. Genetic characterization of smg-8 mutants reveals no role in C. elegans nonsense mediated decay. PLoS One 7, e49490 (2012).
60. Koch, M. A., Haubold, B. & Mitchell-Olids, T. Molecular systematics of the Brassicaceae: evidence from coding plastidic matK and nuclear Chs sequences. Am. J. Bot. 88, 534–544 (2001).
61. Net, M., Xu, P. & Glazko, G. Estimation of divergence times from multiprotein sequences for a few mammalian species and several distantly related organisms. Proc. Natl. Acad. Sci. USA 98, 2497–2502 (2001).
62. Chernikova, D., Matosmed, S., Caiuors, M., Koonin, E. V. & Rogozin, I. B. A late origin of the extant eukaryotic diversity: divergence time estimates using rare genomic changes. Biol. Direct 6, 26 (2011).
63. Yosef, N., Kupiec, M., Ruppin, E. & Sharon, R. A complex-centric view of protein network evolution. Nucleic Acids Res. 37, e88 (2009).
64. Inagaki, Y. & Ford Doolittle, W. Evolution of the eukaryotic translation termination system: origins of release factors. Mol. Biol. Evol. 17, 882–889 (2000).
65. Culligan, K., Tissier, A. & Britt, A. ATR regulates a G2-phase cell-cycle checkpoint in Arabidopsis thaliana. Plant Cell 15, 2651–2664 (2003).
66. Makarova, K. S., Wolf, Y. I., Mekhedov, S. L., Mirkin, B. G. & Koonin, E. V. Ancestral paralogs and pseudoparalogs and their role in the emergence of the eukaryotic cell. Nucleic Acids Res. 33, 4626–4638 (2005).
67. Zmasek, C. M. & Godzik, A. Strong functional patterns in the evolution of eukaryotic genomes revealed by the reconstruction of ancestral protein domain repertoires. Genome Biol. 12, R4 (2011).
68. Cuypers, T. D. & Hogeweg, P. Virtual genomes in flux: an interplay of neutrality and adaptability explains genome expansion and streamlining. Genome Biol. Evol. 4, 212–229 (2012).
69. Wolf, Y. I. & Koonin, E. V. Genome reduction as the dominant mode of evolution. Bioessays 35, 829–837 (2013).
70. Eliaz, M., Klimeš, V., Derelle, R., Petřáleková, R. & Tacheye, J. A paneukaryotic genomic analysis of the small GTPase RABL2 underscores the significance of recurrent gene loss in eukaryote evolution. Biol. Direct 11, 5 (2016).
71. Paterson, A. H. et al. Many gene and domain families have convergent fates following independent whole-genome duplication events in Arabidopsis, Oryza, Saccharomyces and Tetraodon. Trends Genet. 22, 597–602 (2006).
72. Garcia, V. et al. AtATM is essential for meiosis and the somatic response to DNA damage in plants. Plant Cell 15, 119–132 (2003).
73. Culligan, K., Tissier, A. & Britt, A. ATR regulates a G2-phase cell-cycle checkpoint in Arabidopsis thaliana. Plant Cell 16, 1091–1104 (2004).
74. Arciga-Reyes, I., Woottton, L., Kieffer, M. & Davies, B. UPE1 is required for nonsense-mediated mRNA decay (NMD) and RNAi in Arabidopsis. Plant J. 47, 480–489 (2006).
75. Proost, S. et al. PLAZA 3.0: an access point for plant comparative genomics. Nucleic Acids Res. 43, D974–D981 (2014).
76. Vandepoele, K. et al. pico-PLAZA, a genome database of microbial photosynthetic eukaryotes. Environ. Microbiol. 15, 2147–2153 (2013).
77. Grigoriev, I. V. et al. MycoCosm portal: gearing up for 1000 fungal genomes. Nucleic Acids Res. 42, D699–D704 (2013).
78. Camacho, C. et al. BLAST+: architecture and applications. BMC Bioinformatics 10, 421 (2009).
79. Tabach, Y. et al. Identification of small RNA pathway genes using patterns of phylogenetic conservation and divergence. Nature 493, 694–698 (2013).
80. Causier, B., Castillo, R., Xue, Y., Schwarz-Sommer, Z. & Davies, B. Tracing the evolution of the floral homeotic B- and C-function genes through genome synteny. Mol. Biol. Evol. 27, 2651–2664 (2010).
81. Lee, T. H., Tang, H., Wang, X. & Paterson, A. H. PGDD: a database of gene and genome duplication in plants. Nucleic Acids Res. 41, D1152–D1158 (2012).
82. Tang, H. et al. Synteny and collinearity in plant genomes. Science 320, 486–488 (2008).
83. Tang, H. et al. Unraveling ancient hexaploidy through multiply-aligned angiosperm gene maps. Genome Res. 18, 1944–1954 (2008).
84. Linding, R. et al. Systematic discovery of in vivo phosphorylation networks. Cell 129, 1415–1426 (2007).

Acknowledgements
This work was supported by the Biotechnology and Biological Sciences Research Council (to B.D. and B.C.). R.D.S. is a postdoctoral fellow of The Research Foundation–Flanders (FWO), VGD. acknowledges the Multidisciplinary Research Partnership "Bioinformatics: from nucleotides to networks" Project (no. 01MR0310W) of Gent University and funding from the European Union Seventh Framework Programme (FP7/2007–2013) under European Research Council Advanced Grant Agreement 322739 – DOUBLEUP. J.P.B.L. acknowledges the Gatsby Charitable Foundation for funding. Finally, we are grateful to Dr. Mary O’Connell (University of Leeds) for helpful comments during the preparation of the manuscript.

Author Contributions
B.C. conceived the project, wrote and edited the manuscript, produced the main figures and interpreted the data. Z.L. performed data analysis (Suppl T1–3) and edited the manuscript. R.D.S. performed and supervised data analysis (Suppl T1–3) and edited the manuscript. J.P.B.L. performed data analysis (Suppl T5), contributed to and edited the manuscript. Y.V.D.P. supervised the project and edited the manuscript. B.D. supervised the project, contributed to and edited the manuscript.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-017-16942-w.
Competing Interests: The authors declare that they have no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.