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Review

The role of homeodomain transcription factors in fungal development

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ARTICLE INFO

Article history:
Received 29 September 2017
Received in revised form
3 April 2018
Accepted 27 April 2018

Keywords:
Development
Fructification
Homeodomain transcription factors
Multicellularity

ABSTRACT

The role of homeodomain (HD) transcription factors during development in animals is well established since the identification of the homeobox gene clusters. In the kingdom Fungi homeodomain genes also play a crucial role during multicellular development. They were first identified in mating type loci, which regulate sexual development. Later, other HD genes were shown to be involved in fruiting body development in several members of Ascomycota and Basidiomycota. In this review we describe recent research on HD transcription factors in fungi. An evolutionary framework is provided by reanalyzing 222 previously published fungal genomes to identify potential functions of HD transcription factors in multicellular development and fructification.

1. Introduction

Fruiting bodies are among the most complex multicellular structures formed by Fungi and have evolved to produce and disperse spores. Although mushrooms of the order Agaricales are generally best known due to their complex macroscopic structure, fruiting bodies are formed by members of many clades of higher fungi (Ascomycota and Basidiomycota). The genetic regulation of fruiting body formation is not well-characterized, despite its potential economic impact. However, several groups of transcription factors have been identified as regulators of fructification. In the basidiomycete *Coprinopsis cinerea* the light sensing *dst1*, as well as the high mobility group transcription factor *pcc1* have been shown to be involved in fructification (Kamada *et al.*, 2010; Murata *et al.*, 1998). In *Schizophyllum commune* transcription factors containing a zinc finger of the fungal-specific Zn(II)2Cys6 domain (*fst3*, *fst4*), the C2H2 domain (*c2h2*) or the GATA domain (*gat1*, *wc-2*) and transcription factors containing a BRIGHT domain (*bri1*) or a homeodomain (HD) (*hom1*, *hom2*) regulate various aspects of fruiting body formation (Ohm *et al.*, 2013, 2011; Pelkmans *et al.*, 2017). No functional characterization has been done in other Agaricales, although many of the previously mentioned genes are conserved across the Agaricales (Pelkmans *et al.*, 2017).

In the ascomycete *Neurospora crassa* many TF knock-out strains show aberrant perithecium formation: seven Myb-like, seven C2H2, six Zn(II)2Cys6, four GATA, three Zn(II)2Cys6/Fst, two BRIGHT, two bZIP, two homeodomain, two HLH, two HMG, one BRIGHT/Myb-like, one C2H2/Zn(II)2Cys6, one Cbf-NY-Y, one Forkhead, one HTH, KilA-N and one NDT80/PhoG-like transcription factor were shown to have

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https://doi.org/10.1016/j.fbr.2018.04.002

some defect in fructification, ranging from reduced perithecium count to no perithecium formation at all (Aramayo *et al.*, 1996; Carrillo *et al.*, 2017; Colot *et al.*, 2006; Degli-Innocenti and Russo, 1984; Feng *et al.*, 2000; Li *et al.*, 2005).

In the ascomycete *Podospora anserina* five HMG and two homeodomain transcription factors are known to be required for wild type development of the fruiting body (Ait Benkhali *et al.*, 2013; Coppin *et al.*, 2012). In *Aspergillus nidulans* two C2H2, two Zn(II)2Cys6, one Dopey_N leucine zipper, one GATA and one KilA-N transcription factor are identified to be involved in successful conidiophore development (Han *et al.*, 2001; Pascon and Miller, 2000; Prade and Timberlake, 1993; Vallim *et al.*, 2000; Vienken *et al.*, 2005; Vienken and Fischer, 2006; Wu and Miller, 1997).

A homeodomain is a conserved DNA-binding domain of 60 amino acids that features a helix-turn-helix structure. HDs are found in transcription factors in almost all eukaryotic species, except some unicellular species (Derelle *et al.*, 2007). HDs are classified as either TALE (three amino acid length extension) or non-TALE domains, based on the presence of an insertion in the sequence between the two helices. This split occurred before the origin of plants, animals and fungi, as both types are present in each of these kingdoms (Bharathan *et al.*, 1997).

The HD was first identified in homeobox (*hox*) transcription factors in *Drosophila melanogaster* (Lewis, 1978). These *hox* genes are found in several clusters in the genome and control the body plan of an embryo along the anterior–posterior axis (Mallo *et al.*, 2010). For example, a change in the gene order modifies the body plan and leads to the famous antennapedia phenotype, where legs grow in place of the antenna. This is caused by a dominant inversion in the bithorax complex Hox cluster and subsequent *antP* expression in the head (Frischer *et al.*, 1986; Maeda and Karch, 2009). Phenotypical changes such as this led to the name homeodomain, after the process of homeosis, where an organ is replaced by another organ. Similar functions are found in all *Bilateria*, where spatial expression along the anterior–posterior axis determines segment identity by activating specific expression patterns per segment, leading to the development of these segments. As such the localized activation of *hox* genes through developmental pathways ultimately regulates the segment identity in *Bilateria* (Mallo *et al.*, 2010). In plants HD transcription factors play a similarly important role in development (Hay and Tsiantis, 2010). For example, overexpression of the HD transcription factor *kn1* in *Arabidopsis thaliana* leads to lobed leafs, compared to the simple structure in WT plants (Lincoln *et al.*, 1994).

Given their role in developmental processes in a wide range of organisms, it has been suggested that HD transcription factors are part of the genetic toolkit that allowed organisms to develop multicellularity and cell differentiation (King, 2004). In both animals and plants the HD genes expanded to over 100 genes per species (Derelle *et al.*, 2007). In Fungi the diversification happened on a much smaller scale: HD counts range from 1 to 51 (Todd *et al.*, 2014) (this study). Notably, in the kingdom Fungi HDs are also associated with regulating (multicellular) development, since they are involved in mating as well as fruiting body development.

This review will focus on the role of homeodomain transcription factors in multicellular development and fructification in the kingdom Fungi. Recent literature is combined with a re-analysis of 222 previously published fungal genomes to provide a framework for studying the function of HD transcription factors.

2. Homeodomain transcription factors in Fungi

Evolutionary framework for homeodomain transcription factors

In recent years hundreds of fungal genomes have been sequenced and published, including Ascomycota, Basidiomycota and 'early diverging fungi' (i.e. non-Dikarya) (Cerqueira *et al.*, 2014; Cherry *et al.*, 2012; Grigoriev *et al.*, 2014; Hibbett *et al.*, 2016; Nagy *et al.*, 2016; Riley *et al.*, 2016; Skrzypek *et al.*, 2017), resulting in a large number of predicted HD transcription factor genes. These previously published data were reanalyzed and the evolutionary history of fungal HD genes was reconstructed, providing an evolutionary framework for this review (Supplementary Text 1). In the case of Ascomycota and Basidiomycota the number of HD genes generally correlates with the total number of predicted genes (Fig. 1). The early diverging fungi are a clear exception to this, however, and generally have much higher counts of HD genes. This increase can be attributed primarily to the Mucoromycotina (Supplementary Table 1). Furthermore, fungi with a predominantly unicellular (yeast) lifestyle generally have fewer HD genes than fungi with a predominantly multicellular (filamentous) lifestyle (Fig. 2). This suggests that in Ascomycota and Basidiomycota the HD genes tend to be involved with multicellular development (Derelle *et al.*, 2007), although this effect is less pronounced than in the Metazoa (de Mendoza *et al.*, 2013). Interestingly, it was previously reported that the increase in transcription factor count resulting from genome expansion in fungi can be mostly explained by three TF families, including the HD TFs, which show a higher relative increase compared to the TFome as a whole (Shelest, 2017; van Nimwegen, 2003).

A phylogenetic analysis of all predicted HD genes allowed the identification of 12 groups of HD genes (Fig. 3, Supplementary Text 1 and Supplementary Table 1), which were mapped onto the species tree of the 222 fungi (Fig. 4, Table 1). This revealed the evolutionary history of each of these 12 groups of HD genes, which will be discussed below.

Mating type HD transcription factors

The best-studied HD transcription factors in Fungi are those belonging to the mating type locus. In many Fungi this locus is essential for the recognition of (and successful fusion with) a non-self mating partner. This is generally seen as the first step in the initiation of sexual reproduction and (depending on the organism) the associated multicellular differentiation and development (Raudaskoski and Kothe, 2010).

In the ascomycete yeast *Saccharomyces cerevisiae* the mat locus is either mat-a or mat-α and transcription of the locus leads to mat-a or mat-α specific cellular development (Haber, 2012). In mat-α cells, the HD gene *MAT*α*2* is expressed.

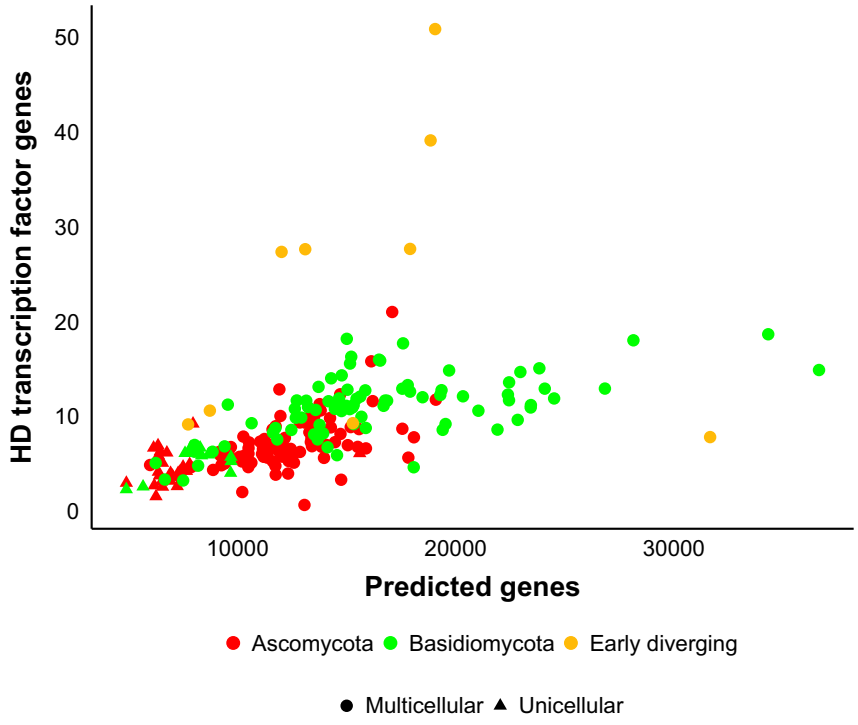

Fig. 1 – Scatterplot of total predicted genes versus predicted homeodomain transcription factors in 222 fungal genomes. The colors indicate the clade and the shape indicates lifestyle (unicellular yeasts versus multicellular filamentous fungi). In members of Ascomycota and Basidiomycota the number of homeodomain transcription factors roughly correlates with the total gene count, but this is not the case for the early diverging fungi. More details can be found in Supplementary Table 1.

Together with Mcm1, MATα2 forms a heterotetramer that represses mat-a specific genes (Elble and Tye, 1991; Keleher *et al.*, 1988; Passmore *et al.*, 1989). This includes mat-a specific pheromone signaling to identify the opposing mating type (Johnson, 1995). In mat-a cells the HD transcription factor *MATa1* is expressed from the mat locus. While *MATa1* is expressed it is quickly degraded and has no known functions

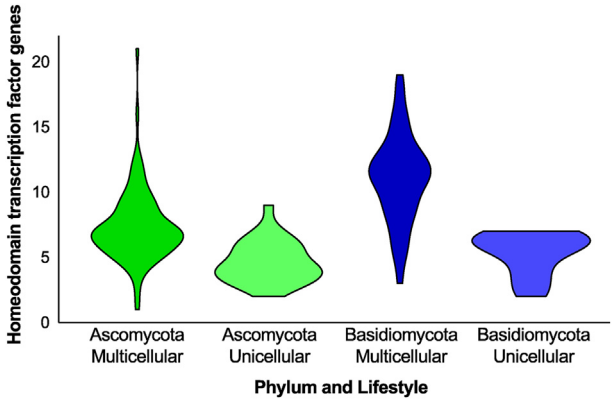

Fig. 2 – The number of homeodomain transcription factors per Phylum and lifestyle (multicellular/filamentous versus unicellular/yeast-like). Unicellular species encode fewer homeodomain transcription factors in their genome when compared to multicellular species. More details can be found in Supplementary Table 1.

in haploid cells, indicating that mat-a development is the default (Johnson *et al.*, 1998). When compatible mating type cells fuse, they create a diploid cell that expresses both *MATa1* and *MATα2*. Expressed together these HD transcription factors form a heterodimer that prevents degradation and has a specificity distinct from the MATα2 homodimer (Goutte and Johnson, 1993, 1988; Keleher *et al.*, 1988). The a1/α2 heterodimer represses a set of genes called haploid-specific genes (Dranginis, 1990). These genes include *MATα1* (*Herskowitz*, 1989), which regulates the haploid mat-α cellular expression and *rme1*, a repressor of meiosis (Covitz *et al.*, 1991). Besides the HD genes, both mat-a and mat-α locus carry another gene: *MATa2* is uncharacterized and is dispensable for successful mating (Dranginis, 1989), while *MATα1* is a HMG box gene responsible for mat-α specific transcription (Bender and Sprague, 1987). A unique feature of several ascomycete yeasts is the ability to switch mating types (Haber, 2012). In *S. cerevisiae*, both upstream and downstream another mating type locus is located, named hidden mat left (HML) and hidden mat right (HMR). The HML locus contains a silenced copy of the *mat-α* genes, while the HMR carries an additional silenced *mat-a* copy. During the late G1 phase a specific endonuclease, HO, can replace the MAT locus with either HML or HMR (Kostriken *et al.*, 1983).

The role of HD transcription factors in mating in Basidiomycota is very similar to that found in *S. cerevisiae*. However, in most Basidiomycota the mating type is determined by two unlinked loci, the *matA* locus for HD transcription factors and the *matB* locus for pheromones and their receptors

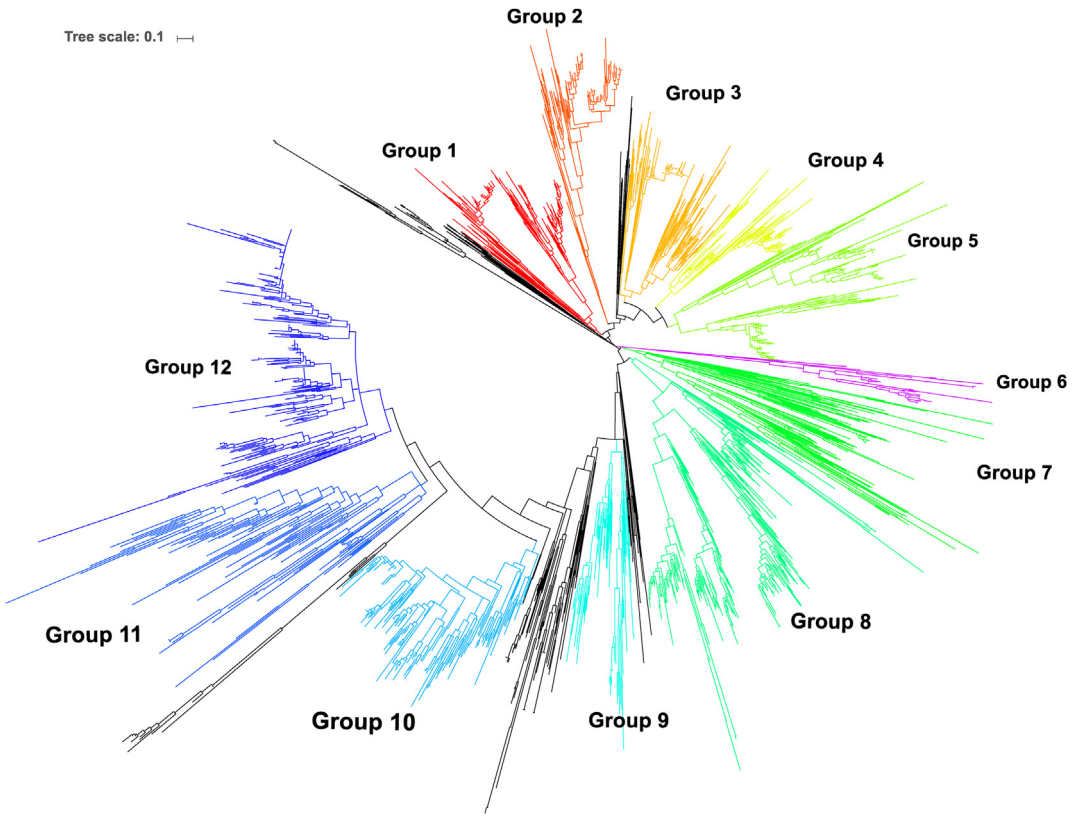

Fig. 3 – Phylogenetic tree of all 2113 homeodomain genes predicted in 222 fungal genomes. Conserved groups of HD genes are indicated in color. More details can be found in Supplementary Table 1.

(Raudaskoski and Kothe, 2010). The presence of two loci makes many Basidiomycota tetrapolar, since meiosis leads to spores with one of four possible mating types. However, in some species, including *Cryptococcus neoformans*, the *matA* and *matB* locus are genetically linked and these species are therefore bipolar (Lengeler *et al.*, 2002). In all heterothallic tetrapolar species a fertile dikaryon is only established when both mating loci are compatible. In most species the *matA* locus is composed of two HD genes, one TALE HD1 and one non-TALE HD2 transcription factor (Kües *et al.*, 1994), although in *C. cinerea* and *S. commune* the *matA* locus is composed of five and six HD genes, respectively (Freihorst *et al.*, 2016; Kües, 2000; Ohm *et al.*, 2010; Stajich *et al.*, 2010). Variation in only a single HD gene is necessary for sexual compatibility. Likely, the expansion of the locus increases the chances of finding a compatible mate in nature and increases genetic variation through genetic recombination. The *matA* HD transcription factors form homodimers or heterodimers (Asada *et al.*, 1997; Ian Robertson *et al.*, 2002; Kües *et al.*, 1994), although heterodimers are necessary for successful mating in *U. maydis* (Gillissen *et al.*, 1992). More complex combinations between homodimerization and heterodimerization have been suggested to occur in Agaricales (Ian Robertson *et al.*, 2002). After dimerization the HD transcription factors facilitate the establishment of clamp connections in dikaryotic mycelium (Raper, 1966). No direct targets of a HD1/HD2 heterodimer have been identified, but in *C. cinerea* it has been suggested that targets include *clp1* and *pcc1* (Raudaskoski and Kothe, 2010), which are both involved in clamp connection formation.

HD transcription factor genes involved in mating are found in group 7 (mating type genes of *S. cerevisiae*), group 9 (HD2 genes of Basidiomycota) and group 11 (HD1 genes of Basidiomycota) (Fig. 3, Table 1 and Supplementary Text 1). None of these groups are closely related to each other, which indicates that these functions likely split early during fungal evolution.

Mating type genes tend to be clustered (co-localized) in mating type loci (Raudaskoski and Kothe, 2010), which is also reflected in the relatively high percentage of clustered genes in these three groups (Table 1 and Supplementary Text 1), particularly in the case of HD1 and HD2 genes in Basidiomycota. This functional clustering of HD genes is a remarkable parallel with the developmentally important *hox* gene cluster in animals (Maeda and Karch, 2009). To our knowledge, similar functional clustering has not been described for any other class of transcription factors.

Ascomycete HD genes involved in fruiting body development

In the ascomycete *P. anserina*, where HD transcription factors are not part of the mating-type locus, seven HD transcription factors were identified in the genome (Coppin *et al.*, 2012). Initial studies on a single HD gene, *pah1*, showed it represses microconidiation and alters hyphal branching (Arnaise *et al.*, 2001). Knockouts of *pah1*, *pah2*, *pah5* and *pah7* showed altered

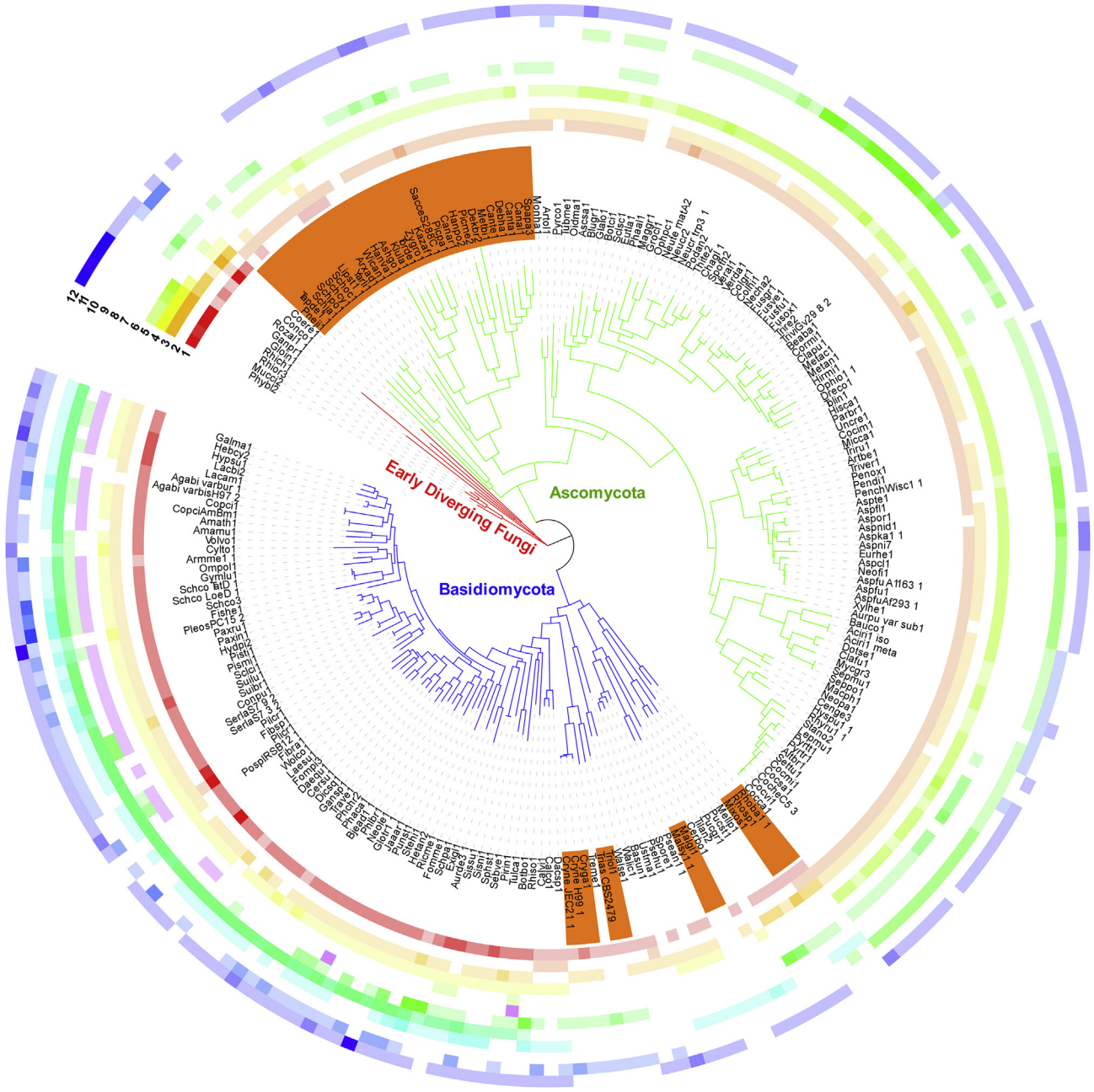

Fig. 4 – Species tree of 222 fungi reconstructed from 57 highly conserved proteins. Subtree color indicates early diverging Fungi (red), Ascomycota (green) and Basidiomycota (blue). Species names in orange have a (mostly) unicellular lifestyle. The heat maps around the tree indicate the number of HD transcription factors in each of the groups identified in Fig. 3. Group 1 is the innermost ring and group 12 is the outermost ring. The heat map gradient colors range from white for no HD transcription factors for that species to five (or more) for the maximum color. An annotation of known genes, functions and conservation for each group is given in Table 1. Species names are abbreviated and more detail can be found in Supplementary Table 1. The branch length of *Hanseniaspora valbyensis* (Hanva1_1) is not drawn to scale. The online tree editor iTOL v3 was used for annotation and visualization (Letunic and Bork, 2016).

perithecium morphology. Particularly, *pah2* and *pah5* knockouts develop fruiting bodies without a neck, which promotes spore dispersal (Ingold, 1933). In both *pah1* and *pah7* knockouts the location of the fruiting bodies on the mycelium was altered and *pah1* knockouts also showed more globular perithecia. In double knockouts it was shown that Pah2 and Pah5 are essential for fructification and sexual reproduction, as Δ*pah2*Δ*pah5* strains did not form any asci and produced only very small fruiting bodies (Coppin *et al.*, 2012). When additional HD gene deletions were introduced, this phenotype

Table 1 – Annotation of the groups of homeodomain transcription factors identified in Figs 3 and 4. More details can be found in Supplementary Table 1 and Supplementary Text 1.

Group	Known proteins	Known functions (inferred from known proteins)	Present in	Type	Gene count	Clustered genes (% of HD genes that are co-localized with another HD gene)
1	*hom1 (S. commune, 257652); hdp1 (U. maydis, 5762)*	Vegetative growth/filamentous growth	Early diverging fungi; Basidiomycota	Non-TALE	208	3.8
2	*yph1/yox1 (S. cerevisiae, YDR451C, YML027W); yox1 (C. albicans, CaO19.7017.1); pah4 (P. anserina, 254); mohox6 (M. grisea, 113426)*	Cell cycle, filamentous growth	Ascomycota	Non-TALE	123	0
3	*hom2 (S. commune, 257987); pah2 (P. anserina, 3203); mohox5 (M. grisea, 114780)*	Fructification/perithecia neck	Early diverging fungi; Pezizomycotina; Basidiomycota	Non-TALE	219	0
4	*hdp2 (U. maydis, 4928)*	Pathogenicity	Early diverging fungi; Agaricomycotina	Non-TALE	118	0.8
5	*Pho2 (S. cerevisiae, YDL106C); Gfr10 (C. abicans, CaO19.4000.1); Pah1/3 (P. anserina, 3360, 1279); kal1 (N. crassa, 7166); mohox1/2 (M. grisea, 116399, 116067)*	Phosphate metabolism, filamentous growth, conidiation	Mucoromycotina; Ascomycota	Non-TALE	209	3.3
6		Unknown	Agaricomycetes	Non-TALE	35	8.6
7	*mata2/alpha2 (S. cerevisiae, YCL067C, YCR039C, YCR096C)*	Mating in Saccharomyces	Saccharomyces; Basidiomycetes (excluding Ustilaginomycetes and Wallemiomycetes); Dothideomycetes	Non-TALE	127	14.2
8		Unknown	Agaricomycetes	Non-TALE	306	11.8
9	HD2 Genes	Mating in Basidiomycota	Basidiomycota (excluding Tremellomycetes and Pucciniomycetes)	Non-TALE	87	83.9
10	*pah6 (P. anserina, 4696); mohox3 (M. Grisea, 119837)*	Unknown	Pezizomycotina	Mixed	175	4
11	HD1 genes	Mating in Basidiomycota	Basidiomycota (excluding Ustilaginomycetes and Puccioniomycotina); Dothideomycetes	TALE	106	67.9
12	*Tos8/cup9 (S. cerevisiae, YGL096W, YPL177C); cup9 (C. albicans, CaO19.6514.1); pah5 (P. anserina, 10096); kal1 (N. crassa, 4379); mohox7 (M. grisea, 111842)*	Meiosis, DNA damage, homeostasis, perithecia neck, pathogenicity	Early diverging fungi; Ascomycota (excluding Schizosaccharomycotina and Dothideomycetes); Basidiomycota (excluding Tremellomycetes)	Mixed	229	10.5

became more pronounced. The model organism *N. crassa* showed similar phenotypes to *P. anserina*, with *pah1* ortholog *kal1* supporting conidium formation and *pah5* ortholog *bek1* involved in the formation of the perithecium neck (Colot *et al.*, 2006). In the plant-pathogenic ascomycete *Magnaporthe oryzae,* HD transcription factors are indispensable for the formation of the appressorium (Kim *et al.*, 2009), a specialized cell used to pierce plant cells upon infection (Liu *et al.*, 2010). Additionally, conidiation is altered or abolished in knock-outs of Δ*mohox2*. Interestingly, *mohox2* is an ortholog of *pah3* in *P. anserina*, knockouts of which did not show any change in phenotype (Coppin *et al.*, 2012). *mohox7* is the ortholog of *pah5* in *M. oryzae*, but is only involved in the formation of the appressorium and not conidiation (Kim *et al.*, 2009). This suggests that homologous proteins do not necessarily have a similar function in different species. However, perithecium formation was not assessed in *M. oryzae* and *mohox7* expression is increased 12-fold during fructification. Similarly, in *Botrytis cinerea*, *pah1* ortholog *bchox8* is also involved in hyphal growth and conidiation (Antal *et al.*, 2012).

Transcriptome data confirms observations of HD transcription factor involvement in perithecium formation *N. crassa*, as *bek1* is initially down-regulated and later up-regulated during fructification (Wang *et al.*, 2014). Expression of *kal1* increases up to four-fold 48 h after initiation of perithecium formation. Several uncharacterized HD genes show similar expression patterns. *NCU03070* and *NCU05257* are both significantly upregulated after 48 h and both *NCU03266* and *NCU09556* are highly expressed in the final stages of fructification (after 120 and 72 h, respectively). Particularly *NCU09556* is of interest as it is an ortholog of the *pah2* in *P. anserina* which has been shown to play a role in fructification (Coppin *et al.*, 2012). *NCU03070* was previously knocked out, but had no phenotype in fructification, asexual development or hyphal growth (Colot *et al.*, 2006). In *Fusarium graminearum* several HD genes are differentially expressed during fructification (Sikhakolli *et al.*, 2012). However, orthologs of *pah1* and *pah2* show relatively low expression and actually decrease during fructification. Interestingly, *Fusarium* species have an expansion of *pah6* homologs almost all of which are upregulated during fructification, like the *N. crassa* ortholog, while *pah6* knock-outs had no observed phenotype (Coppin *et al.*, 2012; Wang *et al.*, 2014). Together this shows the need for functional identification beyond transcriptome analysis to reliably identify genes involved in fructification.

Basidiomycete HD genes involved in fruiting body development

The genome of the basidiomycete *S. commune* encodes 11 non-mating HD proteins, but only two of those have been functionally characterized, *hom1* and *hom2* (Ohm *et al.*, 2011; Pelkmans *et al.*, 2017). The Hom1 transcription factor is involved in mushroom development, maintaining the vegetative state, and increased biomass formation. A *Δhom1* strain exhibits lower biomass formation and reduced hyphal thickness. Moreover, fructification in this strain is increased, but mushrooms are smaller (Ohm *et al.*, 2011). Hom2 is also involved in the fructification process and stimulates fruiting body development (Ohm *et al.*, 2011). The *Δhom2* strain consists of vegetative mycelium and is incapable of fructification. Hom2 is regulated by protein kinase A, which inactivates Hom2 on four RRXS amino acid motifs by phosphorylation (Pelkmans *et al.*, 2017). Mutation of the RRXS-motifs leads to a constitutively active Hom2 transcription factor, resulting in increased fructification. A third HD gene of interest is *hom5* (proteinID: 2603970) and although it has not been functionally studied, it is downregulated during fructification (Pelkmans *et al.*, 2017), suggesting that it plays a role in this process.

No knockouts of HD genes have been reported in other Agaricales, but the *hom1* ortholog in *C. cinerea* (protein ID: 493627) is differentially regulated during fructification, with upregulation in the stipe and downregulation in the cap (Muraguchi *et al.*, 2015). The *hom2* ortholog (protein ID: 355616) is increased in stipe elongation and the later stages of cap development. Together this suggests that similar regulation patterns can be found in multiple Agaricales species. Especially since other regulatory pathways, like light sensing, are also conserved between *S. commune* and *C. cinerea (Kamada et al.*, 2010; Ohm *et al.*, 2013). Additional HD genes with a possible role in development include orthologs of the *S. commune* genes *hom3* (protein ID: 497620), *hom6* (protein ID: 460235) and *hom10* (proteinID: 458752). Expression of *hom3* and *hom10* is down-regulated in later stages of *C. cinerea* development, while *hom6* is differentially regulated depending on developmental stage (Muraguchi *et al.*, 2015).

Yeasts versus multicellular fungi

Yeasts are found in several fungal phyla and include ascomycete species (e.g. *S. cerevisiae, Candida albicans*) and basidiomycete species (e.g. *C. neoformans*) (Nagy *et al.*, 2014). Yeasts are generally less complex than filamentous fungi and may lack the genetic toolkit for complex development due to loss of function during unicellular evolution (Nagy, 2017). Indeed, the genomes of yeasts generally encode fewer HD genes (Fig. 1). Moreover, they have significantly lower HD gene diversification, with one to five groups in Schizosaccharomycotina and Saccharomycotina and two to six groups in Ustilaginomycotina (Fig. 4).

The *Saccharomyces* genome database contains 10 homeodomain entries, including four mating type genes (Cherry *et al.*, 2012; Engel *et al.*, 2014). Among these are the hidden left mat and hidden right mat involved in mating type switching that are not expressed. Other genes include *ste12*, involved in invasive growth and mating (Bardwell *et al.*, 1998), and *pho2*, transcriptional activator of the *pho5*, an acid phosphatase (Liu *et al.*, 2000). Finally, it contains two sets of paralogous genes, *yox1, yph1, tos8* and *cup9*. The former play a role in cell cycling, repressing early cell-cycle boxes (Horak *et al.*, 2002; Pramila *et al.*, 2002), while *tos8* and *cup9* have diversified in different roles. Gene *tos8* promotes meiosis and polarized growth (Horak *et al.*, 2002), while *cup9* reduces epigenetic modifications during heat shock and prevents copper toxicity (Knight *et al.*, 1994). In the ascomycete yeast *C. albicans*, which can also grow hyphae (Sudbery, 2011), *grf10*, a *pho2* ortholog, is important in hyphal growth, with Δ*grf10* strains exhibiting reduced hyphal growth and attenuated virulence (Ghosh *et al.*, 2015). Similar effects have been observed for HD proteins in *C. neoformans*, although they are part of the mating type

locus, and therefore are also considered to play additional roles in development through sexual selection (Loftus *et al.*, 2005; Mead *et al.*, 2015). Nevertheless, it shows how closely related the functions of HD transcription factors can be regardless of evolutionary distance.

Saccharomycotina lack domains related to *pah2* and *pah6* in the filamentous fungus *P. anserina* (groups 3 and 10 in Fig. 4, respectively). The gene *pah2* is implicated in perithecium formation and related to *hom2* in *S. commune*, which is crucial for fructification, so an absence of these groups in yeasts is not unexpected. The function of *pah6* is currently unknown and the lack of homologs in yeasts may indicate a role in multicellularity, although no phenotype was identified in *Δpah6* strains (Coppin *et al.*, 2012).

Basidiomycete yeasts notably lack a group conserved in Agaricomycotina (group 8, Fig. 4), making these HD genes candidates for regulators of the complex development found in Agaricomycotina (*S. commune* protein IDs: 2565648, 2613044, 2693949). However, neither shows differential expression in *S. commune* and *C. cinerea* orthologs are down-regulated during the later stages of fructification (Muraguchi *et al.*, 2015; Pelkmans *et al.*, 2017).

Multicellular ascomycetes have HD genes in four to six groups, while the Agaricomycotina (multicellular basidiomycetes) carry three to ten groups. However, in the latter clade this number varies widely from three to five groups in Tremellales to five to ten groups in Agaricales. Pezizomycotina generally lacked HD2 mating genes, Saccharomycotina mating genes and three Basidiomycete-specific clades. Agaricomycotina lacked Saccharomycotina mating genes, and two Ascomycota clades. Furthermore, the HD domain of *hom2* of *S. commune* is related to *pah2* in *P. anserina*, which is involved in formation of the perithecium neck (Coppin *et al.*, 2012; Ohm *et al.*, 2011). This may indicate that there is overlap in the regulatory pathways involved in ascocarp and basidiocarp formation.

In the early diverging Mucoromycotina considerable gene duplication occurred, with HD gene counts ranging from 23 to 51 (Fig. 1), which is higher than any other fungal species. However, this expansion is confined to five groups of HD genes (Fig. 4). Whole genome duplication has previously been shown in early Mucoromycotina evolution (Corrochano *et al.*, 2016; Ma *et al.*, 2009), but it is currently unknown why the gene count expanded so dramatically.

3. Conclusions and outlook

Recent sequencing efforts have resulted in large numbers of genomes across the fungal tree of life. As a result, the repertoire of homeodomain transcription factors can be easily predicted for each of these fungi. Many of these HD transcription factors play important roles during development in the kingdom Fungi. They are (arguably) best known for their role in mating and subsequent development in both Ascomycota and Basidiomycota. Other HD transcription factors are involved in regulating fruiting body development in several model systems. Not all HD transcription factors are involved in multicellular development, however, as exemplified by their presence in yeasts, albeit in lower numbers. The function of the vast majority of HD genes is currently unknown and this is especially the case for the large expansion of HD genes in early diverging fungi.

Further functional characterization is essential to identify the full role and regulatory network of HD transcription factors. This is hampered by a lack of available molecular tools in many fungi, including protocols for gene knock out. The relatively recent development of CRISPR/Cas9 protocols in several ascomycetes and basidiomycetes may facilitate a more high-throughput approach (Nødvig *et al.*, 2015; Pohl *et al.*, 2016; Qin *et al.*, 2017; Ryan *et al.*, 2016; Sugano *et al.*, 2017).

Regulatory networks involving HD transcription factors can be elucidated using RNA-Seq and ChIP-Seq. While the former is relatively straightforward with the advent of next generation sequencing techniques, the latter is much more laborious. To our knowledge, no direct targets of HD transcription factors have been identified in any filamentous fungus, which would be an important next step to further elucidate the regulatory network. Moreover, only limited data exists on post-translational modification of HD transcription factors and their effect on development. The advent of these (and other) molecular tools may soon improve our knowledge of genetic regulation of development in fungi and this review emphasizes HD genes as important targets.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement number 716132).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.fbr.2018.04.002.

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