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Evolutionary innovation, fungal cell biology, and the lateral gene transfer of a viral KilA-N domain

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Fungi are found in diverse ecological niches as primary decomposers, mutualists, or parasites of plants and animals. Although animals and fungi share a common ancestor, fungi dramatically diversified their life cycle, cell biology, and metabolism as they evolved and colonized new niches. This review focuses on a family of fungal transcription factors (Swi4/Mbp1, APSES, Xbp1, Bqt4) derived from the lateral gene transfer of a KilA-N domain commonly found in prokaryotic and eukaryotic DNA viruses. These virus-derived fungal regulators play central roles in cell cycle, morphogenesis, sexual differentiation, and quiescence. We consider the possible origins of KilA-N and how this viral DNA binding domain came to be intimately associated with fungal processes.

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Introduction

Many Fungi are saprophytic heterotrophs with symbiotic relationships with plants and animals that encompass, and fluctuate between, mutualistic, biotrophic, and necrotrophic interactions. Fungi have general characteristics, such as chitinous cell walls, polar growth to generate long filamentous structures known as hyphae, and elaborate sexual and asexual fruiting bodies to create and disperse spores [1]. Most of these features evolved in the ancestor of Fungi, as it adapted to new ecological niches likely in mutualistic association with early land plants [2]. One source of evolutionary adaptation is gene duplication, which changes the nature of selection on the expanding gene family, thus fueling divergence and the evolution of new functions. However, it is becoming clear that lateral gene transfer (LGT) has played a larger role in eukaryotic evolution than initially thought [3–5]. Here, we focus on a striking example of gene network rewiring in fungi by the LGT of a KilA-N domain from an unknown virus or bacteria [6]. This event happened early in the evolution of fungi and created a large family of KilA-N-containing transcription factors that are associated with core processes in the fungal life cycle (e.g. cell cycle, morphogenesis, sexual differentiation, quiescence). We first consider examples of evolutionary innovation driven by viral and bacterial LGTs before describing the conservation, function, and structure of fungal KilA-N subfamilies. Last, we will speculate on the origins of the ancestral KilA-N domain and how it might have become intimately associated with fungal biology.

LGT as a source of evolutionary innovation in eukaryotes

Mobile DNA elements, viruses, and prokaryotes (e.g. *Agrobacterium*) can contain genes that facilitate the transfer and incorporation of exogenous genetic material into a host genome, whether that be a unicellular eukaryote or the germline of a multicellular eukaryote. Most of these lateral gene transfers (LGT) will become non-functional by deleterious mutation unless they are co-opted by the host to provide some function that keeps them selectively maintained in the genome. Many successful LGTs from viruses to eukaryotes (known as endogenous viral elements, EVEs) have been co-opted by hosts for viral defense [7,8], membrane fusion and vesicle trafficking [9••,10••], or as weapons against other eukaryotes [11]. Some EVEs also play a role in the eukaryotic cell cycle, for example, EBLN1, a bornaviral EVE involved in microtubule organization and DNA-damage repair [12]. The eukaryotic function of these EVEs often mirrors functions (e.g. cell cycle regulation; replication, processing, encapsulation, and transport of nucleic acids) used by existing viruses to propagate and complete their life cycles. This suggests that the ancestral hosts re-purposed an existing function of ancestral viral genes to modulate their own biology.

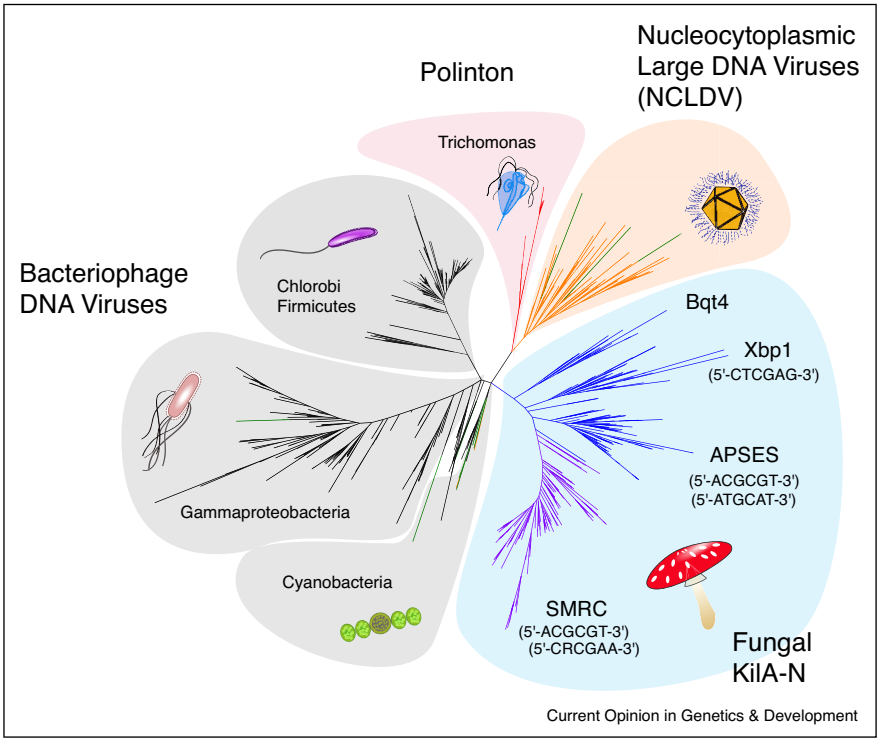
Many prokaryotes can grow in diverse environments because they have an extensive set of metabolic reactions. In contrast, the majority of eukaryotes have relatively reduced metabolic pathways, most likely because their ancestor evolved the ability to engulf organic matter and cells via phagocytosis [13]. The ability to eat cells or

harbor endosymbionts (e.g. bacteria) presents a continuous opportunity for the LGT of bacterial genes into eukaryotic genomes [14]. Thus, it should come as no surprise that many endogenized bacterial genes have been repurposed by eukaryotes to fight bacteria [15] or to create novel metabolic capabilities [16]. The latter spans from the simple to the complex. For example, *HhMAN1* is an endogenized bacterial mannanase gene that allows the beetle *Hypothenemus hampe* to use coffee berry for food [17]. At the other extreme, ciliates and fungi in the rumen of herbivores evolved the ability to ferment plant material using anaerobic respiration by endogenizing hundreds of bacterial genes (e.g. cellulases, hydrogenases) from resident gut bacteria [18,19]. From an evolutionary point of view, bacterial LGT permits naive eukaryotes to co-opt defense genes and metabolic pathways from resident microbial communities that are adapted to their niches. Interestingly, several eukaryotic lineages with osmotrophic lifestyles (e.g. fungi, oomycetes) are conducive to evolution by LGT from prokaryotes and other eukaryotes [5,20]. Although many known fungal LGTs are metabolic in nature, below, we describe an example of a regulatory LGT (e.g. KilA-N domain). This LGT occurred early in the fungal ancestor and evolved into a large transcription factor family that regulates fundamental processes, such as the cell cycle, morphogenesis, quiescence, and nuclear architecture.

LGT of a KilA-N domain and the regulation of fungal cell biology

The KilA gene was first identified in bacteriophage P1 and its expression is lethal to *Escherichia coli* [21], suggesting a bactericidal role. Later bioinformatic work showed that the N-terminal domain of KilA is found in DNA viruses of prokaryotes (e.g. bacteriophage) and eukaryotes (e.g. NCLDV, nucleocytoplasmic large DNA viruses) [22], and as endogenized viral genes in the genomes of bacteria and eukaryotes (e.g. fungi); see Figure 1. Phylogenetic analysis of KilA-N domains was unable to determine whether the endogenized fungal KilA-N domain was an LGT from a eukaryotic virus, prokaryotic virus, or prokaryote [6]. Furthermore, the structure and function of KilA-N domains in prokaryotic or eukaryotic viruses is poorly characterized. Because we know most about the endogenized KilA-N domain in fungi, we will work backwards from the structure and function of SMRC, APSES, Xbp1, and Bqt4 to speculate on the possible origin and evolution of the fungal KilA-N family. Below, we outline how KilA-N is a DNA-binding domain in a family of fungal transcription factors

Figure 1



A large family of fungal transcription factors contains an endogenized KilA-N domain from a eukaryotic virus or a prokaryote.
Cartoon of a maximum-likelihood phylogenetic tree of KilA-N domains from Pfam; see methods in Ref. [6]. There are three main KilA-N clusters: (1) bacteriophage KilA-N and prokaryotes with endogenized bacteriophage KilA-N (grey), (2) nucleocytoplasmic large DNA virus KilA-N (orange) and KilA-N domain in Polinton/Mavericks transposons of *Trichomonas* (red), (3) endogenized fungal KilA-N, which is the DNA-binding domain of the SMRC, APSES, Xbp1, and Bqt4 transcription factor families (blue). The known *cis*-regulatory DNA-binding specificities are listed below each family.

(SMRC, APSES, Xbp1, Bqt4) that regulate cell cycle entry, morphogenesis, quiescence, and nuclear architecture.

SMRC family

This fungal KilA-N family was identified in *Saccharomyces cerevisiae* mutants defective in mating type switching [23–25] and was later shown to regulate cell cycle entry [26–29]. In *S. cerevisiae*, the family has three members, Swi4, Mbp1, and Swi6. Swi4 is a transcription factor that binds the SCB motif (5'-CRCGAA-3'), whereas Mbp1 prefers the MCB motif (5'-ACGCGT-3') in G1/S cell cycle regulated genes. These two transcription factors form heterodimers with a common subunit Swi6 [30,31], a paralog of Swi4/Mbp1 that does not bind DNA in many Hemiascomycetes [32•]. Cell cycle research in *Schizosaccharomyces pombe* identified Res1 and Res2 (orthologs of Swi4/Mbp1) and Cdc10 (an ortholog of Swi6), which forms the basis of the SMRC family name (Swi4/6 Mbp1 Res1/2 Cdc10). Most fungal SMRC, including *S. pombe* [33], bind 5'-ACGCGT-3' indicating that the MCB motif is the ancestral binding preference of this family [32•].

APSES family

The first member of this family (StuA) was isolated from a genetic screen of *Aspergillus nidulans* mutants defective in the development of asexual structures known as conidiophores [34]. Subsequent work isolated homologs and paralogs and showed that they were involved in pseudohyphal growth in *S. cerevisiae* (Sok2, Phd1) [35,36], morphogenesis in *Candida albicans* (Efg1, Efh1) [37], and development of conidiophores in *Neurospora crassa* (Asm1) [38]; see Figure 2. The sequence conservation and shared function of these transcription factors led to the family name, APSES (Asm1 Phd1 Sok1 Efg1 StuA). Similar to Swi4 in SMRC, the binding specificity of APSES changed during the evolution of Hemiascomycetes: The APSES in *S. cerevisiae* and *C. albicans* prefer (5'-ATGCAT-3'), whereas APSES in other fungi bind the ancestral (5'-ACGCGT-3'), which is similar to the ancestral SMRC binding motif. The binding overlap with SMRC and the existing role of APSES in coordinating cell morphology with the cell cycle suggests that this fungal KilA-N family may have evolved to regulate the formation of fungal-specific structures. This is supported by recent bioinformatic work demonstrating that the APSES family is strongly correlated with the emergence of hyphae and multicellularity in the fungal ancestor [39••].

Xbp1 family

This family was identified in *S. cerevisiae* as a paralog of Swi4/Mbp1 [40]. The protein Xbp1 was shown to be a low glucose-activated and stress-activated transcription factor that binds DNA sequence (5'-CTCGAG-3') and represses transcription of target genes, such as the G1/S cell cycle regulator *CLN1*. Subsequent work identified additional gene targets of Xbp1-mediated repression that regulate cell cycle during late sporulation [41], cell morphology during pseudohyphal growth [42], and represses 15% of the yeast genome during the transition to quiescence upon nutrient depletion [43,44]. Many of these repressed genes are involved in cell growth, cell division, and metabolism. This suggests that Xbp1 plays a major role in coordinating the cell cycle and metabolic changes that occur during quiescence and spore formation. Because Xbp1 is as strongly conserved across fungi as the SMRC and APSES subfamilies within Fungi (Figure 2), Xbp1 likely plays a similar role in many other Fungi.

Bqt4 family

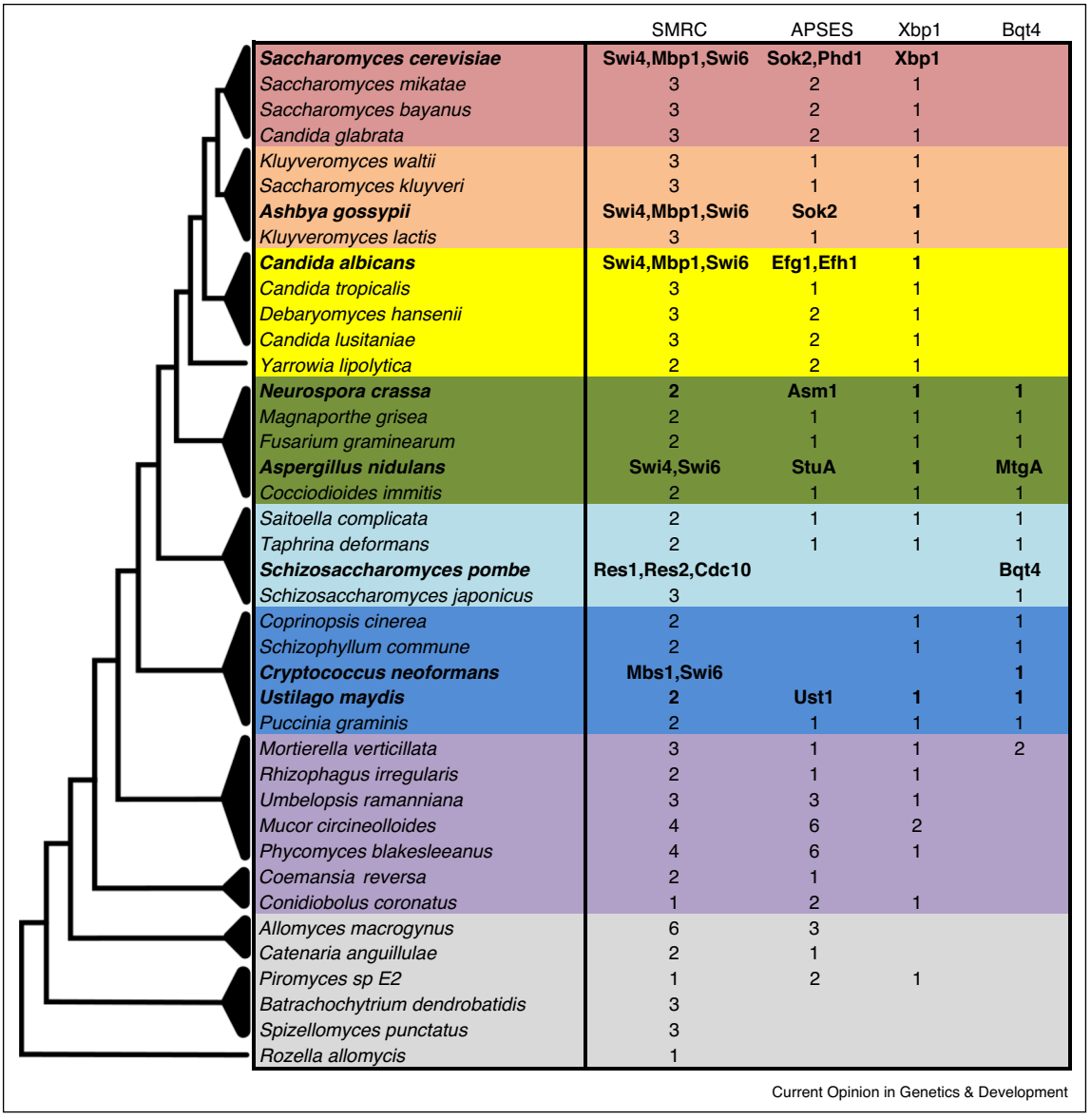
During meiotic prophase, many organisms cluster their telomeres to create a 'bouquet' arrangement of chromosomes. Bqt4 was first identified in *S. pombe* as a protein that is localized to the inner nuclear envelope and helps anchor telomeres during bouquet formation by interacting with telomere-bound Rap1 [45]. A homolog of Bqt4 in *A. nidulans* (MtgA) also localizes to the inner nuclear envelope and plays a role in nuclear and nucleolar architecture during mitosis as well as being required for normal meiosis [46]. Recent work demonstrated that although Bqt4 can bind double-stranded DNA, its binding is relatively weak and non-selective [47••]. From an evolutionary perspective, Bqt4 is the youngest and least distributed member of the fungal KilA-N family (Figure 2).

Structure of the fungal KilA-N family

The DNA-binding domain of StuA was initially suggested to be a basic helix-loop-helix [48]. However, the first structures of a fungal KilA-N domain (Mbp1) showed that the DNA-binding domain contained a helix-turn-helix followed by a long linker region with two anti-parallel beta strands ('the wing'); see Figure 3. These apo-structures were unbound to DNA and the authors relied on computational methods to propose that the recognition helix specifically bound the MCB motif (5'-ACGCGT-3') via the major groove. Later bioinformatic work compared Mbp1 to other structures in the Protein Data Bank and suggested the fungal KilA-N domain had a better alignment with homing endonucleases and tRNA intron endonucleases than to other wHTHs [22].

Two recent KilA-N crystal structures shed light on the mode of DNA binding and structural conservation across fungi. First, Pcg2 (an Mbp1 homolog from *Magnaporthe oryzae* that was crystallized in a DNA-bound form) showed that the wing, and not the helix, binds the minor groove to recognize most of the MCB motif [49]. Second, Bqt4 has an identical structure to that of the SMRC family (Mbp1 and Pcg2) showing that the structure of fungal KilA-N is broadly conserved [47••]. We compared Bqt4 and Pcg2 to other structures using the Dali server [50] and find that fungal KilA-N domains have the best overlap with one another, followed by weaker hits to Orf22 of granulovirus (PDB: 4YE7) and the

Figure 2



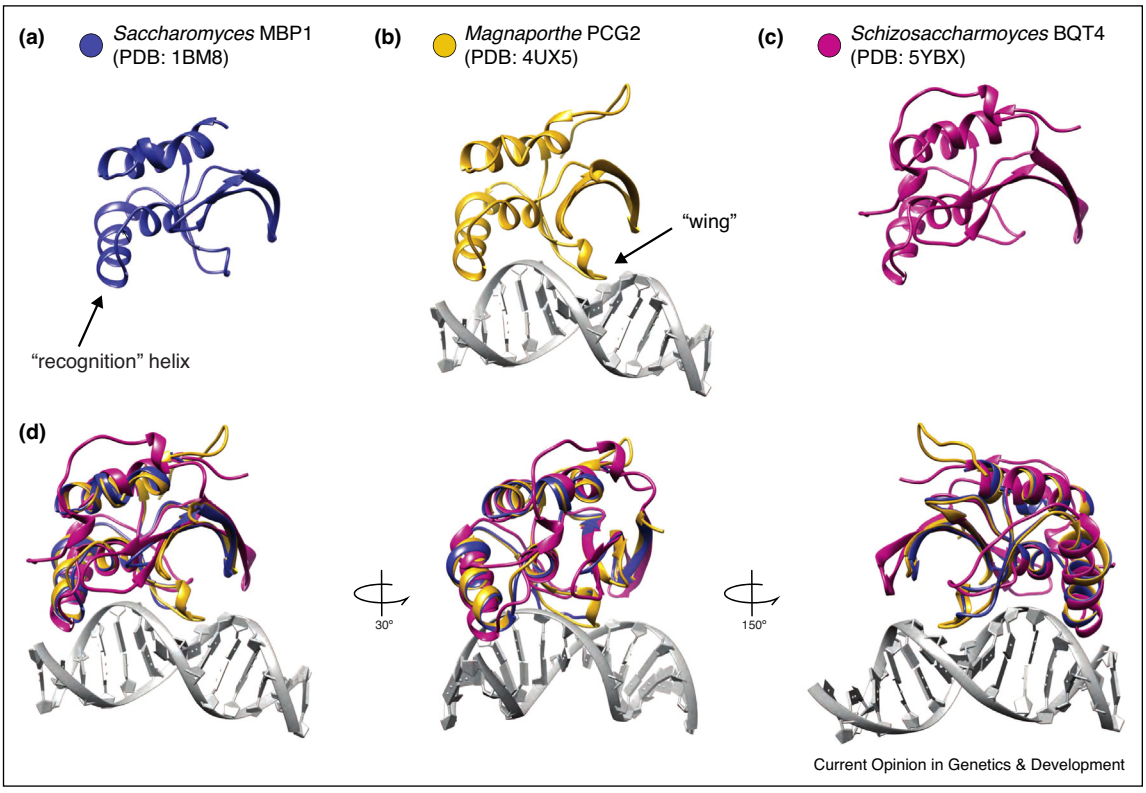
Distribution of fungal KilA-N transcription factor families.
These data are ordered by a coarse species tree (left). The tree includes early diverging fungi (purple, grey) and the Dikarya, which span the Basidiomycota (blue) and Ascomycota, for example, Hemiascomycotina (red, orange, yellow), Pezizomycotina (green), and Taphrinomycotina (cyan). Each column shows the number of KilA-N family homologs detected in the genome. Numbers are replaced with gene names in those species (bold) where the genes have been characterized. This figure was modified from Ref. [6].

forkhead/winged-helix family (e.g. Dachshund, SKI oncogene). Although the evolutionary origin of the KilA-N structure still remains unclear, it seems reasonable to continue classifying fungal KilA-N as a DNA-binding domain with a unique fold that is similar to but distinct from wHTHs. Furthermore, the high level of sequence conservation between viral, prokaryotic, and fungal KilA-N predicts that KilA-N domains will have the same wHTH-related fold and can bind nucleic acids [51].

Origins, function, and evolution of the fungal KilA-N family

The founding member of the fungal KilA-N family was likely a SMRC and the other family members emerged through gene duplication within the SMRC. This is based on (1) the presence and conservation of SMRC in all surveyed fungi (Figure 2), (2) the shared ancestral binding motif (MCB: 5'-ACGCGT-3') of the two largest families (SMRC and APSES), and (3) the fact that the derived APSES and Xbp1 families have maintained a

Figure 3



The fungal KilA-N domain has similarities to a winged helix-turn-helix.
(a) The first structures of Mbp1 (a member of the SMRC subfamily) in *S. cerevisiae* showed a discontinuous, anti-parallel β-barrel structure packed against a bundle of α helices. The resemblance of the conserved DNA-binding domain to a winged helix-turn-helix (wHTH) led the authors to suggest that Mbp1 recognized its specific DNA target (MCB motif: 5'-ACGCGT-3') via a recognition helix in the major groove and that the wing likely made non-specific contacts with the DNA phosphate backbone [53,54]. **(b)** The first protein-DNA structure of KilA-N (Pcg2 from *Magnaporthe oryzae*, homologue of Mbp1/Swi4) showed that the MCB motif is mostly recognized via the minor groove by the wing region (arrow to wing) and that the 'recognition' helix plays a lesser role in MCB specificity [49]. **(c)** A recent structure of Bqt4 from *S. pombe* shows that fungal KilA-N architecture is strongly conserved outside the SMRC family. **(d)** Mbp1 and Bqt4 are aligned and superimposed on Pcg2-DNA structure and viewed from different angles.

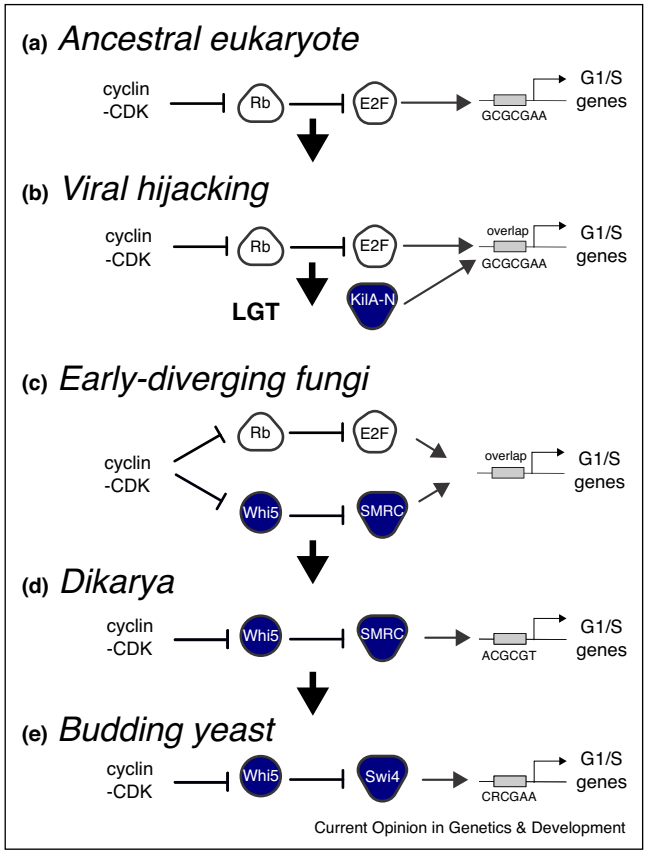
strong connection to cell cycle regulation (the primary function of SMRC) despite having their primary functions in morphogenesis and quiescence.

However, these data say nothing about the viral or prokaryotic origins of the KilA-N domain, which became the putative ancestral SMRC transcription factor. One possible answer comes from an evolutionary analysis of the G1/S regulatory network that regulates cell cycle entry in eukaryotes (Figure 4). Our previous work showed that the ancestral transcription factor E2F, which regulates cell cycle entry in eukaryotes, was replaced by a KilA-N-containing transcription factor (SMRC) in fungi [6]. Ancestral E2F and inhibitor (Rb) and the derived fungal SMRC and inhibitor (Whi5) in budding yeast have analogous mechanisms and architectures that regulate cell cycle entry. Most strikingly, budding yeast Swi4 (an SMRC) has a 5'-CRCGAA-3' binding preference that overlaps the binding preference of ancestral E2F (5'-GCGCGAA-3') [6]. Because endogenized bacterial or viral genes tend to maintain their original function in their new host, we speculate that ancestral KilA-N was transferred from a eukaryotic virus. Similar to oncogenic genes in viruses that push eukaryotic cells into a proliferative state, the function of viral KilA-N may have been to bind E2F-regulated genes and deregulate the ancestral G1/S regulatory network that controlled cell cycle commitment. This viral KilA-N gene was subsequently endogenized as the founding member of the SMRC and co-opted into the fungal G1/S regulatory network, such that the ancestral E2F pathway could be lost in the ancestor of the Dikarya (Figure 4). The SMRC transcription factor further expanded and diversified through gene duplication to create the APSES, Xbp1, and Bqt4 families that regulate fungal morphogenesis, quiescence, and nuclear architecture.

Future directions

Understanding how a KilA-N domain was endogenized and co-opted by the ancestor of fungi could help address

Figure 4



A viral KilA-N domain hijacked the cell cycle by binding E2F sites and was later endogenized as SMRC.
(a) The ancestral G1/S regulatory network (top) contains an E2F transcription factor that is inactive during the G1 cell cycle phase by the binding of retinoblastoma (Rb) inhibitor. When conditions are favorable for cell cycle entry (e.g. nutrients, pro-growth signals), Rb is phosphorylated by cyclin-CDK and loses its ability to inhibit E2F, such that G1/S genes are expressed and the cell irreversibly commits to another round of the cell division cycle. **(b)** A eukaryotic viral KilA-N domain with overlapping specificity to E2F sites hijacked the cell cycle, similar in principle to oncogenic viruses that deregulate the G1/S transition. **(c)** The fungal ancestor likely evolved a Whi5 inhibitor to keep the former KilA-N domain (an endogenized SMRC transcription factor) suppressed during the G1 cell cycle phase. This may have been followed by phospho-entrainment of Whi5 by cyclin-CDK to create a functionally redundant G1/S regulatory pathway. This hybrid network exhibits partial and interspersed preservation across the early diverging fungi, consistent with the expected evolutionarily instability of redundant pathways [6]. Neither the hybrid network nor the binding affinity of E2F or SMRC have been characterized in early diverging fungi. **(d)** The ancestral E2F-Rb pathway was completely lost in the transition to Dikarya and functionally replaced by the SMRC-Whi5 pathway. The ancestral SMRC binding motif 5'-ACGCGT-3' has overlapping binding specificity with E2F. **(e)** Subspecialization in DNA-binding preference of Swi4 in budding yeast. This figure was modified from Ref. [6].

the evolutionary mechanism by which other regulatory LGTs might rewire highly conserved networks and processes. Concomitantly, dissecting how a KilA-N domain evolved into a family of fungal-specific transcription factors that regulate cell cycle, morphogenesis, and quiescence, should provide clues on the selective pressures and adaptations that drove the emergence of the fungal kingdom. Tackling these issues would require two complementary approaches. A bottom up approach would continue characterizing the mechanisms and function of KilA-N-containing SMRC, APSES, Xbp1, and Bqt4 within fungi, especially early diverging fungi. On the other hand, a top down approach would characterize the evolution and function of KilA-N domains in other eukaryotes, viruses, and even prokaryotes. These approaches would help distinguish between alternative functions for ancestral KilA-N. For example, ancestral KilA-N could have been Xbp1-like and regulated quiescence in the host cell (similar in spirit to other viruses [52]), which then expanded and diversified to create SMRC, APSES, and Bqt4. The development of new genetic and experimental tools in deep lineages of eukaryotes, including early fungi, along with the sequencing of inaccessible eukaryotic genomes (and their viruses, symbionts, and microbiomes) from the environment would help elucidate the role of LGT in eukaryotic evolution.

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Conflict of interest statement

Nothing declared.

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