Introduction

The five heterotetrameric Adaptor Protein (AP or adaptin) complexes function in cargo-selection and coat-recruitment stages of vesicular transport in eukaryotic cells. Much of what we know about AP complex function has come from experimental work using Saccharomyces cerevisiae as a model. Here, using a combination of comparative genomic and phylogenetic approaches we provide evolutionary context for the knowledge gained from this model system by searching the genomes of diverse fungi as well as a member of the sister group to all fungi, Fonticula alba, for presence of AP subunits. First, we demonstrate that F. alba contains all five AP complexes; whereas, similar to S. cerevisiae, most fungi retain only AP-1 to 3. As exceptions, the glomeromycete Rhizopaghus irregularis maintains a complete AP-4 and chytrid fungi Spizellomyces punctatus and Batrachochytrium dendrobatidis retain partial AP-4 complexes. The presence of AP-4 subunits in diverse fungi suggests that AP-4 has been independently lost up to seven times in the fungal lineage. In addition to the trend of loss in fungi, we demonstrate that the duplication that gave rise to the β subunits of the AP-1 and AP-2 complexes in S. cerevisiae occurred before the divergence of F. alba and Fungi. Finally, our investigation into the AP complement of basal fungi (Microsporidia and Cryptomycota) demonstrates that, while the cryptomycete Rozella allomyces contains an adaptin complement similar to other fungi, the extremely reduced Microsporidia retain, at most, a single cryptic AP complex in the absence of clathrin or any other putative AP-associated coat protein.

From all to (nearly) none
Tracing adaptin evolution in Fungi

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Keywords: Adaptins, AP complex, membrane trafficking, evolutionary cell biology, Microsporidia, clathrin mediated endocytosis, fungal evolution

Abbreviations: AP, adaptor protein or adaptin; BLAST, basic local alignment search tool; CCVs, clathrin coated vesicles; CME, clathrin mediated endocytosis; COP, coat protein; HMM, hidden Markov model; TGN trans-Golgi network

The five adaptor protein (AP) complexes function in cargo-selection and coat-recruitment stages of vesicular transport in eukaryotic cells. Much of what we know about AP complex function has come from experimental work using Saccharomyces cerevisiae as a model. Here, using a combination of comparative genomic and phylogenetic approaches we provide evolutionary context for the knowledge gained from this model system by searching the genomes of diverse fungi as well as a member of the sister group to all fungi, Fonticula alba, for presence of AP subunits. First, we demonstrate that F. alba contains all five AP complexes; whereas, similar to S. cerevisiae, most fungi retain only AP-1 to 3. As exceptions, the glomeromycete Rhizopaghus irregularis maintains a complete AP-4 and chytrid fungi Spizellomyces punctatus and Batrachochytrium dendrobatidis retain partial AP-4 complexes. The presence of AP-4 subunits in diverse fungi suggests that AP-4 has been independently lost up to seven times in the fungal lineage. In addition to the trend of loss in fungi, we demonstrate that the duplication that gave rise to the β subunits of the AP-1 and AP-2 complexes in S. cerevisiae occurred before the divergence of F. alba and Fungi. Finally, our investigation into the AP complement of basal fungi (Microsporidia and Cryptomycota) demonstrates that, while the cryptomycete Rozella allomyces contains an adaptin complement similar to other fungi, the extremely reduced Microsporidia retain, at most, a single cryptic AP complex in the absence of clathrin or any other putative AP-associated coat protein.

Introduction

The five heterotetrameric Adaptor Protein (AP or adaptin) complexes function as cargo adapters that recruit coat proteins during vesicle formation at various stages and locations in the membrane trafficking system. AP-1 mediates formation of clathrin coated vesicles (CCVs) that traffic between the trans-Golgi network (TGN) and early endosomes. AP-2 recruits clathrin to the plasma membrane and is involved in vesicle formation during clathrin-mediated endocytosis (CME). AP-3 is involved in vesicle transport between tubular endosomes and lysosomes. AP-4 is involved in vesicle transport from the TGN to endosomes and the cell surface and has been linked to human disorders such as Alzheimer’s disease, cerebral palsy, and spastic paraplegia. The recently discovered AP-5 complex has also been linked to Alzheimer’s disease and spastic paraplegia. AP-4 has been shown that Vps41, a protein with a clathrin heavy chain domain, likely plays a role similar to clathrin in AP-3 vesicles. While the coat protein for AP-4 vesicles is currently unknown, two human proteins (SPG11 and SPG15) interact with AP-5, are structurally similar to clathrin, and thus likely perform a homologous function in AP-5-mediated processes.

AP complexes are homologous to the coat protein complex COPI, with both structural and functional similarities retained. COPI comprises two large subunits (γ and β-COP), a medium subunit (δ-COP) and a small subunit (ζ-COP). Likewise, each AP complex comprises two large subunits (γ1, α2, β3, ε4, ζ5, and β1–5) a medium subunit (μ1–5) and a small subunit (σ1–5). Since all five AP complexes as well as COPI have been shown to have eukaryote-wide distribution, it is thought that the paralogous expansions that gave rise to these complexes all occurred before the divergence of the major eukaryote lineages. Thus, it can be inferred that all five

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Submitted: 12/23/13; Revised: 02/03/14; Accepted: 02/04/14
Citation: Barlow LD, Dacks JB, Wideman JG. From all to (nearly) none: Tracing adaptin evolution in Fungi. Cellular Logistics 2014; 4:e28114; http://dx.doi.org/10.4161/cl.28114
AP complexes were present in the Last Eukaryote Common Ancestor (LECA).

Although all five AP complexes were present in the LECA and are widely distributed across extant eukaryote diversity, each AP complex (except AP-1) has been lost in various lineages. The clade of salivarian trypanosomes including Trypanosoma brucei has been shown to have lost AP-2 but retain clathrin and maintain a modified version of CME. AP-3 has been lost in two independent lineages of Apicomplexa. AP-4 has been lost several times: in Fungi, e.g., S. cerevisiae; several kinetoplastids e.g., the Leishmania genus, Phytomonas serpens, as well as Trypanosoma conglobens; in diplomonads, e.g., Giardia intestinalis; and in the Archaeplastida in both red algae, e.g., Cyanidioschyzon merolae, and green algae, e.g., Chlamydomonas reinhardtii. AP-5 is the most often lost adaptin complex and has been lost at least once in every major group of eukaryotes (See Hirst et al. for further details). All eukaryotes investigated thus far retain clathrin except the intracellular parasites, the Microsporidia. Remarkably, the microsporidian Encephalitozoon cuniculi was reported to lack clathrin but maintain AP subunits.

Microsporidia are extremely divergent, extremely reduced, intracellular pathogens. Due to their apparent lack of

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Figure 1. Coulson plot showing clathrin and adaptin subunits in Fungi and F. alba. Homologs of adaptin and clathrin components were found using a combination of BLAST and HMMer algorithms. Presence of identified protein sequences is represented by a filled-in pie piece: failure to identify a candidate ortholog represented by an open piece. The tree is based on the topology obtained by James et al.
mitochondria and Golgi, they were once considered to be basal, early-diverging eukaryotes. With the discovery of extremely reduced mitochondria called mitosomes, and Golgi-homologs in Microsporidia, this idea has been abandoned. Phylogenetic advances have now firmly placed Microsporidia in the fungal kingdom; thus, instead of being basal eukaryotes, microsporidia now represent perhaps the most divergent and reduced of all known eukaryotes. Reduction of other systems and processes in microsporidia has been investigated (e.g. metabolic pathways, transporter proteins, mitochondrial protein import pathways, genome content); however, the extent to which AP and clathrin loss has occurred in diverse microsporidia is currently unknown. Thus, in this study we investigate the evolution of AP complexes in the fungal lineage with a particular focus on the enigmatic intracellular parasites, the Microsporidia.

**Results and Discussion**

The last common ancestor of Holomycota contained a complete set of adaptins

Since some animals (e.g., humans) retain all five currently identified AP complexes, it can be inferred that the last common ancestor of animals also had at least five AP complexes. However, since the diversity of fungal genomes now available has not been systematically analyzed for presence of AP complexes the complement of AP complexes present in the last common fungal ancestor remains unknown. Knowing the complement of AP complexes in the fungal ancestor would allow us to trace the pattern of AP loss from the fungal ancestor across the fungal tree of life to the more familiar taxa in the Dikarya (e.g., Cryptococcus neoformans and S. cerevisiae). Thus, in order to identify the complement of AP complexes likely present in the fungal ancestor we searched the recently sequenced genome of Fonticula alba, a member of the fongiculids, the sister-group to Fungi. Using BLAST and HMMer homology searching algorithms we identified a complete set of adaptins in *F. alba* (Fig. 1). The only subunit that we could not confidently identify was β3 for which we found a putative homolog whose identity could not be confirmed by reciprocal homology searches. However, since only β1, β2, and the putative β5 sequences were retrieved by our original HMMer analysis we think that this sequence could represent an extremely divergent β5 subunit. All other *F. alba* adaptin subunits were verified by reciprocal homology searching into either the *Homo sapiens* or *Thecamonas trahens* genomes for reference. Since animals are known to contain all five AP complexes, the presence of all five AP complexes in *F. alba* indicates that the ancestor of Fungi and Fonticula retained the full adaptin complement present in the LECA and likely had a more complex endosomal trafficking system than that seen in *S. cerevisiae*.

**Multiple loss of AP-4 in the fungal tree of life**

Having established that the ancestral holomycotan had all five AP complexes, we wanted to determine the distribution of AP complexes across the known diversity of fungi. It has been previously shown that *S. cerevisiae* has maintained AP-1 to 3. However, the extent to which adaptin complexes are retained in other fungi has not been systematically assessed. As above, we used BLAST and HMMer homology searching algorithms, to search the genomes of representatives from relevant fungal lineages (see James et al. including Ascomycota, Basidiomycota, Glomeromycota, Mucoromycotina, Entomophthoromycotina, Kickxellomycotina, Blastocladiomycota, Chytridiomycota and Neocallimastigomycota. We found homologs of all AP-1 to 3 subunits in representatives from each of these major clades (Fig. 1). The only subunit we could not identify was ε3 from *Piromyces* sp., a member of the Neocallimastigomycota. Since ε subunits are very short, failure to detect could be explained by extreme sequence divergence or an incompleteness of the database.

Since it had been previously reported that the basidiomycete *C. neoformans* has retained ε4 but the ascomycete *S. cerevisiae* has not, we were interested in uncovering the extent to which AP-4 subunits have been retained in other fungi. Our homology searches confirmed that no AP-4 subunits are detectable in *S. cerevisiae* or any other ascomycete (Fig. 1); and furthermore, while we confirmed the previously reported presence of ε4 in *C. neoformans*, we could not find evidence for the presence of any other AP-4 subunits in *C. neoformans* or any other basidiomycete analyzed in our study. No AP-5 subunit was detected in any ascomycete or basidiomycete genome. The presence of ε4 in *C. neoformans* indicates that the ε4 subunit has been lost independently in at least two basidiomycete lineages. It is unknown what role a lone ε4 might play in endosomal trafficking in *C. neoformans* and is thus an attractive candidate model for studying degenerate AP function.

Next, we searched for AP-4 and AP-5 in basally diverging fungi (fungi other than ascomycetes and basidiomycetes) to determine the pattern of AP loss in less familiar taxa. While we could not find any evidence for AP-5 in any genome analyzed, in searching the genome of the glomeromycete, *R. irregularis*, we found a complete AP-4 complex (Fig. 1). AP-4 subunits were also identified in the chytridiomycete *Spizellomyces punctatus* (ε) and *Batrachochytrium dendrobatidis* (ε and σ). No AP-4 subunits could be detected in genomes sampled from other fungal lineages including Mucoromycotina, Entomophthoromycotina, Kickxellomycotina, Blastocladiomycota, and Neocallimastigomycota, suggesting that AP-4 has been lost numerous times over the course of fungal evolution. In fact, if the currently accepted fungal tree is correct, then AP-4 has been independently lost seven times.

**AP-1 β and AP-2 β were present in the last common ancestor of the Holomycota**

The only AP subunits not present in the LECA were β1 and β2. Instead, the LECA contained a single β1/2 protein that likely interacted with both AP-1 and AP-2 complexes. This is evidenced by phylogenetic analyses and the presence of only a single β1/2 protein in several extant organisms spanning the tree of eukaryotes including *Drosophila melanogaster*, *Caenorhabditis elegans*, *Monosiga brevicollis*, *Phytophthora ramorum*, *Dictyostelium discoideum* and *Oryza sativa*. In our investigation of the adaptin complement of Fungi we noticed that every genome analyzed contained two β1/2 proteins (Fig. 1). Since the *S. cerevisiae* β1/2 proteins Applp and Ap2lp have been determined to act independently in AP-1 (β1 = Ap2lp) and AP-2 (β2 = Ap1lp), we wanted to determine if these *S. cerevisiae* proteins are the
result of an ancient duplication in Holomyxota, or if multiple lineage-specific duplications have occurred. In order to answer this question, we reconstructed the phylogeny of holomycotan β1–4 subunits (Fig. 2). Our analysis robustly reconstructed the β2, β3, and β4 clades. Although the clade designated as β1 was not well supported in our analysis, it was strongly excluded from...
all other clades. In addition, since no β1 protein was ever robustly placed within the β2 clade it can be inferred that the β1 proteins did not arise from independent lineage-specific duplications. Taken together, these results indicate that the β1 proteins most likely form a monophyletic clade resulting from a single duplication event involving the ancient β1/2 protein. Thus, it can be concluded that the common ancestor of Holomycota had both β1 and β2 subunits that arose from a single ancient duplication of the ancestral β1/2 protein.

That the β1/2 duplication occurred early in the evolution of the holomycotan lineage is rather surprising. In Holozoa (multicellular animals and their protistan relatives), the sister lineage to Holomycota, many basal taxa including the choanoflagellate M. brevicollis as well as several invertebrates like C. elegans and D. melanogaster, contain only a single β1/2 subunit. This suggests that the duplication seen in animals occurred rather late in the course of animal evolution (i.e., in the vertebrate lineage). Similarly, in the excavate lineage, while the heterolobosean Naegleria gruberi has a single β1/2 subunit, the kinetoplastids have undergone a duplication and thus have both β1 and β2. As more genomes become available, it will become possible to determine precisely when β1/2 duplications occurred in lineages like excavates, plants, and animals (see Dacks et al.36).

**Microsporidia lack clathrin but retain (cryptic) adaptin subunits**

After analyzing AP complexes in other fungi, we wanted to determine the adaptin complement present in the most basal clade of fungi that comprises Microsporidia and Cryptomycota. Using alignments generated from our set of identified fungal AP components we constructed HMMs and searched for adaptins in the genomes of the cryptomycete Rozella allomyces and several microsporidians. In similar findings to most other fungi, we identified AP-1 to 3 subunits in the R. allomyces genome but could not identify any AP-4 subunits. Since only the δ3 and µ3 subunits could be found in the R. allomyces genome it is possible that AP-3 may be undergoing a process of degeneration. However, our inability to identify the other AP-3 subunits could again reflect extreme sequence divergence or the incompleteness of the database rather than genuine loss. As more cryptomycete genomes are sequenced, further investigation will determine the extent of AP loss in this lineage.

Microsporidia have been suggested to lack clathrin but retain AP subunits. In our analysis of microsporidian genomes we detected, at most, a single representative of each of the four AP subunits (Fig. 3). The only exception was the presence of two closely related µ subunits in Nosema apis which, based on

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**Figure 3.** Coulson plot of adaptin subunits identified in cryptomycetes and microsporidians. Homologs of adaptin and clathrin components were found as described in Figure 1. The tree is based on topologies obtained in previous studies. Question mark indicates ambiguity of complex identity.
reciprocal BLAST analysis into the *N. apis* genome, appear to be the result of a relatively recent gene duplication or gene database error (data not shown). While clathrin is in all other fungi (Fig. 1), neither the clathrin heavy nor light chain was detected in any microsporidian genome analyzed (Fig. 3). Of note, a single complete set of AP subunits, presumably an AP complex, was detected in several species across the diversity of microsporidia including *Encephalitozoon cuniculi*, *E. intestinalis*, *N. bombycis*, *Vittaforma cornaeae*, *Enterocytozoon bieneusi*, *Trachipleistophora hominis*, *Edhazardia aedis*, *Antonospora locustae* and *Vavraia*

**Figure 4A.** Concatenated phylogenetic analyses of Holomycotan AP complex subunits. The microsporidian sequences are orthologs of AP-1 or AP-2, with AP-1 being the slightly preferred hypothesis. Concatenation of β, γαδ, and σ subunits of AP-1 to 4 shows robust support for grouping of the microsporidian clade with the AP-1 and 2 clades.
culicis floridensis. Three of the four AP subunits were detected in *N. apis* and *N. ceranae* (each was missing a different AP subunit). Only two subunits were detected in *Spraguea lophii* (μ and σ); and remarkably, only a β subunit could be detected in the basally diverging nematode parasite *Nematocida parisiis*. Microsporida are notorious for having extremely divergent protein sequences. Therefore, failure to detect could be caused by extreme sequence divergence; thus, our hypothesis of loss must be followed-up with experimental studies. As new genomes are sequenced, and potentially more basal microsporidians are discovered, more light will be shed on the evolutionary history of reduction in these enigmatic parasites.

The cryptic AP complex of Microsporida is likely either AP-1 or AP-2

Thus far, we have demonstrated that a single AP complex is present in several microsporidian species; however, the identity of this complex, at this point, remains unclear. Since clathrin is not present in microsporidia, we reasoned that if another coat could be found, its presence could provide further clues to the identity of microsporidian adaptins. However, while coat proteins like Vps41 and Spg11 were detected in other genomes (Spg11 was only found in *F. alba*), these proteins could not be detected in any microsporidian genome analyzed (data not shown) and could not provide information on the identity of the cryptic microsporidian adaptins.

Thus, we searched for other ways to determine the identity of the cryptic AP complex. Homology searching experiments using microsporidian AP protein sequences as queries retrieved adaptin subunits from AP-1 to 4 with approximately equal E-values, providing little guidance as to evolutionary provenance of the microsporidian AP complex (data not shown). We therefore reconstructed the phylogenies of each AP subunit from a set of basal fungi in an attempt to determine the identity of the microsporidian adaptins (Figs. S1-S4). Unfortunately, these trees were largely uninformative as microsporidian sequences either did not resolve into a particular AP clade (Figs. S1 and S2), or were robustly excluded from all AP clades (Fig. S3). Microsporidian μ subunits were excluded from AP-1, 2, and 4 μ clades but were not strongly excluded from the AP-3 μ clade (Fig. S4) raising the possibility that the microsporidian μ proteins are μ3. However, our analysis of μ alignments revealed the conservation of a tryptophan residue that is present in all fungal μ1 and μ2 proteins but absent in μ3 and μ4 proteins. This residue is also conserved in nearly all microsporidian μ proteins (not shown). Therefore, although our single-protein μ phylogeny places the microsporidian μ proteins in a clade with fungal μ3 proteins, we suggest that this is likely an artifact due to long branch attraction. Instead we support the more likely hypothesis that the microsporidian AP subunits are all derived from a single AP complex.

Since the single-protein phylogenies did not show robust discordance, and assuming that the four microsporidian AP subunits are derived from a single AP complex having a shared evolutionary history, we chose to use a concatenated phylogeny of AP subunits in order to increase the phylogenetic signal and resolve the identity of the microsporidian AP complex. However, to err on the side of caution, we excluded μ from our concatenated analysis in case it is indeed μ3, although we believe this to be unlikely. Our concatenated phylogeny provided a tree with excellent support (Fig. 4A), uniting the microsporidian AP with the clades of AP-1 and AP-2. Since the topology of the concatenation is similar to the topology seen in the γδε phylogeny (Fig. S3), we hypothesized that the γδε phylogenetic signal was over-powering the phylogenetic signal in the β and σ proteins. Thus, we performed a concatenated phylogenetic analysis of only the β and σ subunits in order to get a more resolved phylogeny for these two subunits (Fig. 4B). This analysis provided robust exclusion of the microsporidian proteins from all AP clades except AP-1. Due to the identification of the conserved tryptophan in the μ1/2 and microsporidian μ proteins, as well as the β and σ single protein and concatenated phylogenies, we conclude that it is likely that the microsporidian complex is most likely either AP-1 or AP-2. Given that AP-1 has never been reported as lost in any eukaryote, and given the result of the β and σ concatenated tree, it is tempting to suggest that the microsporidian complex is derived from AP-1. However, this is admittedly highly speculative and requires functional confirmation.

If this conclusion is correct, the question that arises is: How does AP-1 or AP-2 function in the absence of clathrin? Fortunately, there are some examples in the literature that can help answer this question. First, in the apicomplexan *Toxoplasma gondii* clathrin acts at the TGN in conjunction with AP-1; however, clathrin is not present at the plasma membrane. Instead, AP-2 functions at the plasma membrane without clathrin. Therefore, if *T. gondii* AP-2 can function without clathrin, then it is conceivable that the microsporidian AP complex may function similarly. Second, although microsporidia contain COPI and COPII it has been suggested that they do not contain true COPI or COPII vesicles; instead, membranes are proposed to be trafficked progressively from the ER through the Golgi compartment to the plasma membrane without vesicles. If this is true, then the microsporidian AP complex could potentially function similarly to microsporidian COPI, but at the TGN-like compartments.

**Conclusion**

In this study, we have provided context to AP evolution in fungi that will allow for better application of knowledge gained from experimental models like *S. cerevisiae* to fungal diversity as a whole. First, we explored the genome of *F. alba* and demonstrated that the last common ancestor of the Holomycota contained all five AP complexes. Second, our analysis has shown that several independent losses of AP-4 have occurred over the course of fungal evolution. In our analysis, only the glomomycte *Rhizophagus irregularis* was found to retain a complete AP-4 complex. Thus, this species is a candidate model organism for studying AP-4 function in fungi. Third, in addition to the general trend of loss in fungi, our results indicate that the duplication that gave rise to the β1 and β2 proteins in *S. cerevisiae* occurred very early, prior to the divergence of *F. alba* and Fungi. This means that, although functional data from β1 and β2 in *S. cerevisiae* cannot be directly compared with the similarly named genes in metazoan systems, there are direct orthologs of these genes in other fungi that can, and should, be compared.
This opens new avenues of investigation of membrane-trafficking in the alternate model systems in fungi such as *Neurospora, Yarrowia* and more. Last, we present evidence that microsporidia contain an extremely reduced endosomal system, as they lack clathrin and retain, at most, only a single cryptic AP complex.

Our results demonstrate the ubiquity of AP-1 to 3 in fungi suggesting a general conservation of endosomal trafficking throughout this kingdom. This suggests that findings about these complexes in *S. cerevisiae* are likely generalizable to other fungi. However, presence of AP-4 subunits in several fungi...
highlights lineages where generalizations from *S. cerevisiae* are not adequate. Furthermore, in highly reduced organisms like the Microsporidia where only a single cryptic AP complex is retained knowledge gained from model systems is not very informative. Thus systems that contain a more complex (*R. irregularis* and *F. alba*) or more reduced (Microsporidia) membrane trafficking than familiar model organisms represent important avenues for further experimentation.

**Methods**

**Genome databases**

Publicly available genomes analyzed in this study include: from the Joint Genome Institute: *Aspergillus nidulans*, *Tuber melanosporum*, *Yarrowia lipolytica*, *Rhodotorula graminis*, *Atractiellales* sp., *Cryptococcus neoformans*, *Ustilago maydis*, *Rhizophagus irregularis*, *Rhizopus oryzae*, *Conidiobolus coronatus*, *Coenamia reversa*, *Catenaria anguilulae*, *Batrachochytrium dendrobatidis*, *Piromyces sp.*, *Rozella allomycis*, *Antonospora locustae*; from the Broad Institute Microsporidia Comparative Sequencing Project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/): *Fonticula alba*, *Spizellomyces punctatus*, *Allomyces macrogynus*, *Nematocida parisii*, *Varraia culcis floridensis*, *Vitaforma cornea*, *Edhazardia aedis*, *Nosema ceranae*, *Encephalitozoon cuniculi*, *Encephalitozoon intestinalis*; from NCBI: *Neurospora crassa*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Spraguea lophii*, *Enterocytozoon bieneusi*, *Nosema bombycis*, *Nosema apis*.

**Homology searching**

Putative AP orthologs were identified using a modified reciprocal best hit method. First, previously identified opisthokont AP-1 to 4 subunits were used to construct Hidden Markov Models (HMMs) of all four complex subunits. AP-5 subunit HMMs were constructed separately using a set of previously validated sequences found by Hirst et al. These HMMs were then used to search for homologs in various holomycotan genomes using HMMer (http://hmmer.janelia.org/). A reciprocal pHMMer search into the human genome was then performed using HMMer with the LG model. Divergent sequences representing long branches were removed from the alignments of initial data sets (data not shown) in order to limit the effects of long-branch attraction. The microsporidian sequences were aligned separately from those of other fungi in the final data sets, in order to reduce the effect of random alignment of unconserved positions. The number of taxa and amino acid positions in the alignments for all figures are shown in Table S2, and all alignment files are available by request.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

We would like to thank the members of the Dacks lab for discussion, as well as K. James, B. Kopetski, C. Lavergne, S. McCormack, N. Ellis and C. Thulin for organizational and administrative support. This work is supported by an NSERC Discovery Grant and a New Faculty Award from Alberta Innovates Technology Futures to JBD. JBD is the Canada Research Chair in Evolutionary Cell Biology.

**Supplemental Material**

Supplemental Material may be downloaded here: www.landesbioscience.com/journals/cellularlogistics/article/28114.
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