Chapter 15
Evolution of Fungi and Their Respiratory Metabolism

Marina Marcet-Houben and Toni Gabaldón

Abstract The oxidative phosphorylation (OXPHOS) pathway plays a central role in the energetic metabolism of aerobic organisms. Despite such centrality, this pathway has not remained unaltered through evolution, and variations of it, including its complete loss, can be found in organisms adapted to different ecological niches. Fungi, a eukaryotic group of species with a high metabolic diversity, represent an ideal phylum in which to study the evolutionary plasticity of the OXPHOS pathway from a phylogenomics perspective. With more than 100 completely sequenced genomes, and thanks to recent progress in elucidating their evolutionary relationships, fungal species have served to reveal the evolutionary mechanisms that underlie the evolution of the core respiratory pathways. In this chapter, we review recent progress toward the characterization of OXPHOS components in fungi and in understanding their evolution. A special focus is devoted to the history of duplications that the multi-protein complexes in OXPHOS have experienced.

15.1 Introduction

The fungal kingdom, one of the eukaryotic groups with the highest number of fully sequenced genomes (http://www.genomesonline.org), comprises a large diversity of species, including mushrooms, yeasts, and molds. The exact number of species is unknown but estimates set this value around 1.5 million (Hawksworth 1991), being 700,000 a conservative, lower estimate (Schmit and Mueller 2007). Therefore, only between 5% and 10% of the total diversity of fungi has been characterized so far (Mueller and Schmit 2007). Fungi are ubiquitous and are able to colonize a broad

M. Marcet-Houben • T. Gabaldón
Comparative Genomics Group, Bioinformatics and Genomics Programme, Centre for Genomic Regulation (CRG), UPF, Doctor aiguader, 88. 08003, Barcelona, Spain
e-mail: mmarcet@crg.es

P. Pontarotti (ed.), *Evolutionary Biology – Concepts, Biodiversity, Macroevolution and Genome Evolution*, DOI 10.1007/978-3-642-20763-1_15,

diversity of habitats. This high ecological plasticity has been driven by parallel changes in their basic metabolism, resulting in a broad diversity of metabolic capacities found across fungal lineages.

15.1.1 Adaptation of the Respiratory Pathway in Fungal Species

While most fungal species are aerobic, some lineages have adopted alternative modes of respiration. A clear example is, for instance, the adaptation of some yeast species to fermentation and to anaerobic lifestyles. Adaptation to anaerobic environments can sometimes be very high. An early study performed by Visser et al. (1990) found that some fermentative strains were able to grow even under strict anaerobic conditions (i.e., *Candida tropicalis* or *Saccharomyces cerevisiae*). Although in most cases growth rates were impaired, species closely related to *S. cerevisiae,* were able to maintain near optimal growth rates. While all species that have the ability to grow under anaerobic conditions are fermentative, the opposite is not true. Species such as *Debaryomyces hansenii* or *Pachysolen tannophilus* are fermentative and yet unable to grow under strict anaerobic conditions. Filamentous fungi also have mechanisms to obtain energy when oxygen is limited. Two such mechanisms are nitrate respiration (also known as denitrification) and ammonia fermentation. These mechanisms have been thoroughly studied, for instance, in the filamentous fungi *Fusarium oxysporum* (Takaya 2009). Under limited oxygen conditions, *F. oxysporum* obtains energy from the denitrification pathway by reducing nitrate to nitrous oxide. When the amount of oxygen decreases further, *F. oxysporum* switches to ammonia fermentation, reducing nitrate to ammonia.

Two extreme adaptations to anaerobic conditions are illustrated by two groups of basal fungi: Chitrids and Microsporidians. Chitrids are anaerobic fungal species that contain hydrogenosomes instead of regular mitochondria (Voncken et al. 2002). Although initially considered to be different organelles, it is now clearly established that hydrogenosomes evolved from mitochondria (Hackstein et al. 2006). Hydrogenosomes are able to use protons as electron acceptors and generate hydrogen, acetate, and carbon dioxide. On the other hand, Microsporidia contain yet another form of highly derived mitochondria, the so-called mitosomes. Mitosomes are organelles devoid of DNA and which completely lost all components of the OXPHOS pathway (Burri et al. 2006). It is likely that these species only have the ability to produce energy via glycolysis and that they prefer to import ATP from the host (Williams et al. 2010). Remarkably, genome analysis of the microsporidian *Enterocytozoon bieneusi* shows a complete lack of the glycolitic pathway in this intracellular parasite, illustrating extreme dependence on energy provided by the host (Keeling et al. 2010).

There is plenty of evidence that all fungi have a mitochondria or related organelle and even if the ability for aerobic respiration has been lost in some of the early diverging fungal groups, it is safe to assume that the last common ancestor of fungi was an aerobic organism with a fully functional OXPHOS pathway that has

since undergone changes in a lineage specific manner, thereby enabling the adaptation of fungi to a diverse variety of ecological niches.

15.1.2 Clinical Relevance of Fungal Respiratory Metabolism

Numerous fungal species are pathogenic. They can be commensals, becoming pathogenic when the host is immunocompromised (e.g., *Candida glabrata* or *Candida albicans*), or they can reside in the environment and only become pathogenic when they enter in contact with their host (as it is the case with *Histoplasma capsulatum* or *Cryptococcus neoformans*). The inner tissues of the hosts are usually oxygen-poor, therefore adaptation to anaerobic lifestyles as discussed above can be particularly helpful for survival of the fungal pathogen during infection. This adaptation is extreme when the mode of parasitism is intracellular. Microsporidia are obligate intracellular parasites that are unable to survive outside their host unless it is in the form of spores. Such strict host-dependent lifestyle does not require aerobic respiration, and as a consequence these species have lost the entire OXPHOS pathway.

Adaptation to poor oxygen levels in plant and animal pathogens should be parallel to adaptation to high oxidative stress. Although this may seem contradictory, it is explained by the fact that oxidative burst, the rapid increase of reactive oxygen compounds, is a common defense mechanism against infection. The objective of this defense mechanism is to degrade pathogenic organisms that enter certain plant tissues or that have been internalized by phagocytes in animals. As a defense, many fungi have an alternative respiratory mechanism. The alternative oxidase bypasses complexes III and IV and greatly reduces the reactive oxygen compounds generated by the fungal cell during respiration. This, coupled with enzymes that are able to reduce the reactive oxygen species generated by the host, increases the chance of survival of fungi during infection. For instance, *Aspergillus fumigatus* (Magnani et al. 2008) is able to survive the host's defense mechanisms and continue with the infection once it has escaped the macrophages.

15.1.3 Industrial Relevance of Fungal Respiratory Metabolism

Fungi have been traditionally used in the elaboration of food and beverages such as bread, cheese, wine, sake, or beer. Similarly, fungi were the source for the first antibiotics (penicillin). Nowadays they are still used in industries related to these and other products such as enzymes and acids, and it is clear that the interest in the biotechnological potential of fungi continues to grow. Some of the species that are more widely used in industry are the filamentous fungi *Aspergillus* or *Penicillium* and the yeasts *S. cerevisiae* and *Pichia pastoris* (Li et al. 2010). For instance, citric acid is used widely in the food and pharmaceutical industries. Before 1923, this

compound was produced from lemons. Nowadays, this practice has been replaced by the use of *Aspergillus niger* to synthesize citric acid. Fungi are also often used for the synthesis of antibiotics. One example is cyclosporin, which can be isolated from *Tolypocladium inflatum* (Suvase et al. 2010). This compound was first used as an antifungal, but later, it was shown to possess immunosuppressive activity. Cyclosporin A is currently the most widely used drug for preventing rejection of human organ transplants.

In a biotechnological setting, fungal species are often placed in bioreactors and subjected to over-oxygenation. This can lead to oxidative stress, defined as the overproduction of reactive oxygen species (ROS), which cells cannot defend against with their antioxidant defenses. ROS is generated in mitochondria during the process of aerobic respiration. Fungal species under oxidative stress conditions may suffer morphological changes, a slow growth rate, a low substrate consumption rate, low protein, and ATP content. This last consequence can be attributed to the switch from normal respiration to alternative respirations. In such situations, alternative pathways can substitute parts of the electron transport chain, lowering the production of ROS compounds. However, these alternative routes are unable to translocate protons, therefore the amount of ATP derived from the OXPHOS pathway is severely reduced.

15.1.4 The Oxidative Phosphorylation Pathway

The OXPHOS pathway is used by aerobic organisms to obtain energy. It can be found in all the domains of life with very few exceptions. In most eukaryotic organisms, the pathway is formed by five multi-subunit complexes that work coordinately to produce energy in the form of ATP (Joseph-Horne et al. 2001). The four first complexes (NADH: ubiquinone oxidoreductase, succinate dehydrogenase, ubiquinol cytochrome c reductase, and cytochrome c oxidase) in the pathway form the electron transport chain. This chain starts with the transference of electrons from NADH or $FADH_2$ to ubiquinone, a task carried out by complexes I and II. The next step, performed by complex III, transfers the electrons from ubiquinol to cytochrome c. Finally, complex IV transfers the electrons to molecular oxygen. Electron transfer in complexes I, III and IV, is coupled with the translocation of protons across the inner mitochondrial membrane, creating a proton gradient. The energy produced during the release of the proton gradient is used by complex V in order to produce ATP. The pathway is located in the mitochondrial inner membrane, and, at least under certain circumstances, the complexes are associated into supramolecular structures called respirasomes (Wittig et al. 2006).

Some of the subunits of the OXPHOS pathway are encoded in the mitochondrial genome. Phylogenetic studies have tried to establish the origin of the different components of the pathway and while some nuclearly encoded subunits have a clear mitochondrial origin, the phylogenies of other genes are much more complex (Gabaldon and Huynen 2003, 2004; Gabaldon et al. 2005).

15.1.5 Alterations to the Main Pathway in Fungi

Beyond the common electron transport chain, in plants, fungi and several protists, there are alternative pathways that can bypass some steps. These alternative pathways have conferred a better adaptation for some fungal species and produced fail-safe mechanisms that act when the organism is under stress or toxic conditions.

15.1.5.1 Alternative NADH Dehydrogenase

In contrast to mammals, plants, fungi, and bacteria can bypass complex I with alternative types of NADH dehydrogenases (Kerscher 2000). The main functional difference between alternative NADH dehydrogenases and complex I is that the electron transport to ubiquinone by alternative NADH dehydrogenases is not coupled to proton translocation. In addition, they use FAD or FMN as prosthetic groups and work either as monomers or homodimers.

Alternative NADH dehydrogenases can act either alone, substituting complex I, or in tandem with the regular complex. In *S. cerevisiae*, for instance, complex I has been lost and in its place three different alternative NADH dehydrogenases can be found. One of them faces the matrix while the others face the cytoplasm and compensate for the absence of the malate/aspartate shuttle in this microorganism. In baker's yeast, the alternative NADH dehydrogenases are single polypeptides of 53 or 58 kDa (external and internal, respectively) and are devoid of iron–sulfur centers (Helmerhorst et al. 2002).

These three alternative NADH dehydrogenases can also be found in *Neurospora crassa*, which, unlike baker's yeast, does contain complex I. It has been suggested that in this case, the alternative dehydrogenases are used to prevent the over-reduction of electron-transport carriers and the production of reactive oxygen species. Numerous studies have been performed in order to elucidate the function of these enzymes in *N. crassa*. For instance, it was seen that the internal NADH dehydrogenase, while not essential for growth or sexual development, affected the germination of ascospores and conidia (Duarte et al. 2003).

Numerous human mitochondrial diseases are associated with defects in the functionality of complex I such as Leigh syndrome or Parkinson's disease (Yagi et al. 2006). In recent years, the inner alternative NADH of *S. cerevisiae* (NDI1) has been considered as a potential treatment for those diseases. It has been shown in mouse models that once NDI1 is imported into the cells it can be expressed and is functionally active, and that it remains active for at least several years (Marella et al. 2009). Thanks to the great structural differences between NDI1 and complex I, the former is only one peptide while complex I is formed by around 40 different subunits, this enzyme is totally insensitive to the compounds that can damage complex I.

15.1.5.2 Alternative Oxidase

Alternative oxidases can be found in numerous fungal species, higher plants, algae, and some protozoa, though its distribution within these groups is patchy. Their main function is to serve as an alternative pathway for the completion of the electron transport chain. They bypass complexes III and IV by transferring electrons from ubiquinol to the final acceptor, O_2. They are located in the inner membrane of mitochondria and confer resistance against toxic compounds such as cyanide, for which complex IV is sensitive.

As with alternative NADH dehydrogenases, AOX are unable to translocate protons when electrons are transferred, therefore no energy is produced in this step. It would be extremely unlikely to find alternative NADH dehydrogenases and AOX working together as that would imply a futile cycle. For this reason, it is not surprising to observe that fungal species that lack complex I have also lost the alternative oxidase.

Surprisingly, functional copies of AOX have been found in some anaerobic Microsporidians (Williams et al. 2010). These enzymes have a high quinol oxidase activity and it is thought that they are used in order to lower the reducing potential created through continuous use of glycolysis. Even so, the distribution of AOX in Microsporidians remains patchy and is missing in completely sequenced genomes such as *Encephalitozoon cuniculi*, *E. bieneusi*, or *Nosema ceranae*.

15.2 Establishing the Evolutionary Framework: The Fungal Species Tree

In order to trace the evolution of OXPHOS components across sequenced fungal species, we first need to establish a reliable scenario depicting their evolutionary relationships. In other words, we need to reconstruct a fungal species tree as well as to evaluate the level of confidence of the inferred lineages. Efforts to reconstruct fully resolved phylogenies have been much influenced by the availability of completely sequenced genomes. In particular, methods based on tree concatenation and supertree strategies (Delsuc et al. 2005) have been extensively used at different taxonomic levels (Snel et al. 2005; Wolf et al. 2001). The large amount of available sequence data for fungal species has opened the doors for the use of phylogenomics to address the reconstruction of the still elusive fungal species tree.

Several species trees have been reconstructed over the last few years based on completely sequenced genomes (Marcet-Houben and Gabaldon 2009; Fitzpatrick et al. 2006; Wang et al. 2009). These trees, while slightly different in the distribution of some species, mostly support a similar topology (Marcet-Houben and Gabaldon 2009).

In Fig. 15.1, we present the largest tree that has been reconstructed to date based on phylogenomics data. The tree represents the evolution of 102 different fungal

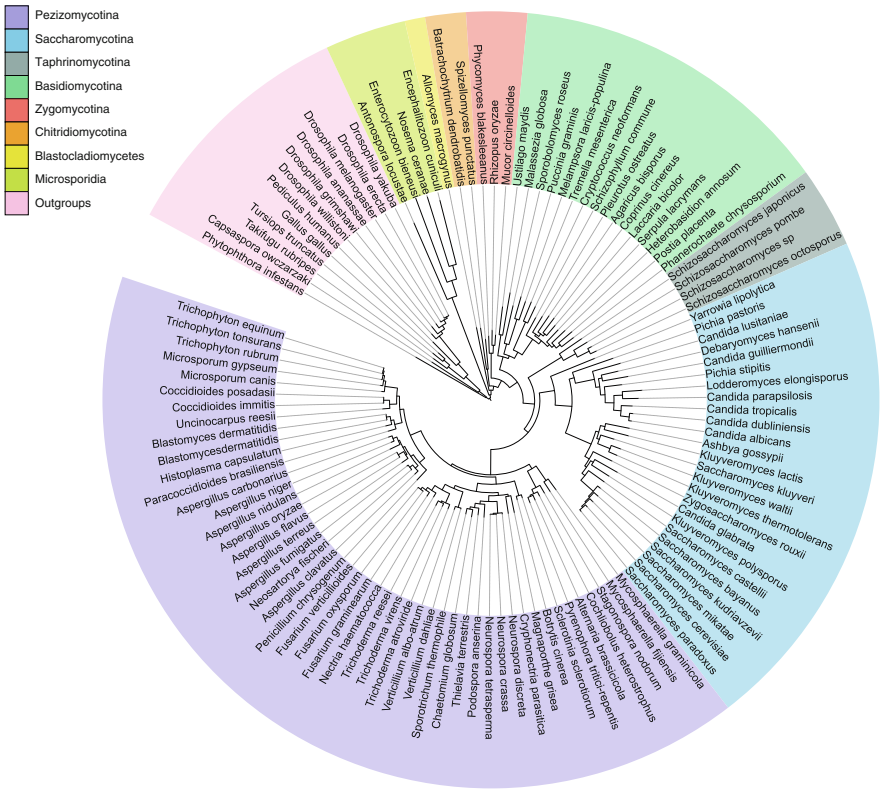

Fig. 15.1 Species tree representing the evolution of fungal species. In order to reconstruct the tree, 47 widespread proteins in 103 fungal species were concatenated and then the maximum likelihood tree was reconstructed. Representation was done using iTOL (Letunic and Bork 2007)

species and is based on the concatenation of 47 widespread proteins that complied with two conditions: they displayed a one-to-one orthology relationship and they were present in at least 90 species. Out-groups were chosen so that the number of proteins used was maximized. The resulting topology is largely similar to the ones published before. Still, some nodes present variability, such as the branching order of the three groups of Basidiomycota or the relative position of *C. glabrata* and *Saccharomyces castellii* in reference to the *Saccharomyces* group.

Traditional methods such as the bootstrap measure (Felsenstein 1985) or the approximate likelihood ratio rest (aLRT) (Anisimova and Gascuel 2006) have been used to determine the robustness of the nodes in a tree. Unfortunately, trees derived from gene concatenation tend to have high support values independently of the tree robustness. New methods are therefore needed to assess the robustness of nodes in the species tree. The phylome support, as implemented in treeKO (http://treeko.cgenomics.org), is one such method. A phylome is the complete collection of phylogenetic trees based on each gene in a genome and this large

collection of trees can be used as a tool to identify nodes that are not well supported by the information encoded in the genome. For each node in the tree, the phylome support algorithm groups species derived from the node into groups and then considers all the arrangements of the groups. Then, the support of each arrangement within the phylome is calculated. The more supported it is, the more often it will appear in the phylome. Nodes with a low phylome support are the ones that we will need to consider carefully while studying the evolution of the OXPHOS pathway.

15.3 Evolution of the Oxidative Phosphorylation Pathway in Fungi

The OXPHOS pathway shows an enormous evolutionary plasticity. Therefore, it is of great interest to see what changes have occurred and relate them to the different respiratory phenotypes in extant fungal species. Lavin et al. (2008) performed a first study by detecting homologs of OXPHOS components in 27 fully sequenced fungal species. We later extended this study to encompass a total of 60 completely sequenced species (Marcet-Houben et al. 2009). An important addition to our study was the reconstruction of the phylogenetic trees of all OXPHOS components across the 60 species under study. Such phylogenetic approach allowed us to reliably distinguish between orthology and paralogy relationships and to investigate the relative timing and taxonomic scope of the gene duplications that have affected OXPHOS components. Gene duplication is considered to be one of the main sources for functional diversification (Ohno 1970), and there are specific hypothesis that predict evolutionary constrains to such mode of evolution in multi-protein complexes (Papp et al. 2003). Thus, we wanted to investigate to which degree this process had affected a group of families mostly coding for large multi-protein complexes and, in principle, expected to retain their central functions. The details of this study can be found in (Marcet-Houben et al. 2009), but we will offer here a brief overview.

We started our search for homologs with a representative from each OXPHOS pathway family, preferably from a model species such as *S. cerevisiae*, *N. crassa*, or *C. albicans*. By searching against a database comprising 60 completely sequenced species, we formed initial groups of homologs that were subsequently curated manually to remove spurious hits or add homologs that had been missed due to annotation errors or methodological artifacts. Finally, these homologs were aligned and a Maximum Likelihood approach (Guindon and Gascuel 2003) was used to infer the phylogenies for each of these groups. By using a species-overlap algorithm (Huerta-Cepas et al. 2007), speciation and duplication events were mapped onto the tree to define orthology and paralogy relationships. This data was used to derive a phylogenetic profile for each family, comprising information about the number of gene copies present in each genome. The main findings of this study are

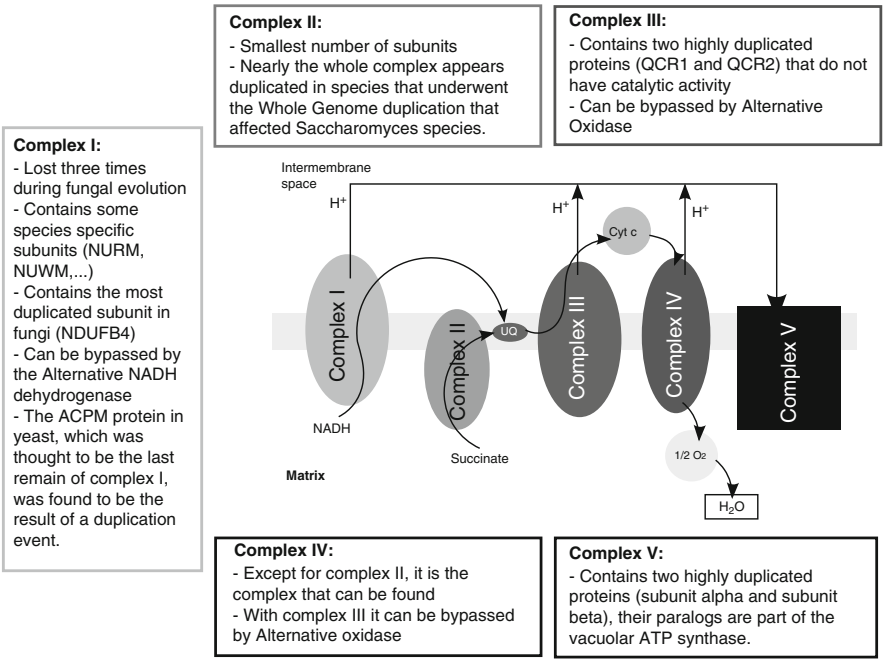

Fig. 15.2 Representation of the main oxidative phosphorylation pathway and the main findings for each of the five complexes that conform the pathway

summarized in Fig. 15.2. We will now center our attention to some of the details that came forth during the study.

15.3.1 Presence/Absence Pattern of the Fungal OXPHOS Genes

In Table 15.1, we show the percentage of fungal species that contain each subunit of the OXPHOS pathway. Darker colors represent widespread proteins while lighter colors represent genes that can only be found in some fungal groups. For instance, the lighter color found in all the proteins in complex I shows that the entire complex was lost in some fungal species. It is known that complex I was lost independently in three lineages, namely, in *Saccharomyces* species, in *Schizosaccharomyces* species, and in Microsporidia, which is confirmed by our analysis (Gabaldon et al. 2005). Thanks to the information provided by the fungal species tree, we can infer the relative timing (i.e., after which speciation events) of each of the three independent losses.

On the other hand, while almost all the proteins of the other complexes can be found in a high percentage of the fungal species, there are some proteins that have a limited taxonomic scope. For instance, NUVM and NUWM were at first considered

Table 15.1 Table of subunits that form the oxidative phosphorylation pathway. Cells are colored according to the presence rate of each subunit. The presence rate is calculated as the number of species that contain at least one copy of the subunit divided with the total number of species considered in the analysis. *Darker colors* represent higher presence rates and decrease in the following intervals: 1.0–0.9–0.75–0.5–0.33–0

Presence rates							
Complex I							
1.6.5.3	NAD1	NAD2	NAD3	NAD4	NAD4L	NAD5	NAD6
NDUFA1	NDUFA11	NDUFA12	NDUFA13	NDUFA2	NDUFA4	NDUFA5	NDUFA6
NDUFA8	NDUFA9	NDUFAB1	NDUFB3	NDUFB4	NDUFB7	NDUFB8	NDUFB9
NDUFS1	NDUFS2	NDUFS3	NDUFS4	NDUFS6	NDUFS7	NDUFS8	NDUFV1
NDUFV2	NI9M	NURM	NUVM	NUWM	NUXM	NUZM	–
Complex II				Alternative NADH			
SDHA	SDHB	SDHC	SDHD	dehydrogenase		Alternative oxidase	
Complex III							
CytB	CytC	ISP	QCR1	QCR10	QCR2	QCR6	QCR7
QCR8	QCR9	–	–	–	–	–	–
Complex IV							
COX1	COX10	COX11	COX15	COX17	COX2	COX3	COX4
COX5	COX6	COX6A	COX6B	COX7	COX8	–	–
Complex V							
SUB 8	SUB A	SUB α	SUB B	SUB β	SUB C	SUB D	SUB δ
SUB ε	SUB F	SUB G	SUB γ	SUB H	SUB J	SUB K	SUB OSCP

to be specific for the OXPHOS pathway in *Yarrowia lipolytica* (Abdrakhmanova et al. 2004). Now, it has been seen that they are not so restricted in their taxonomic sampling. NUWM orthologs can be found in all the members of the Candida group while NUVM is extended to most Ascomycotina species.

There is a possibility that some of the proteins that have a restricted species distribution belong to the same protein family but that due to accelerated evolution, we are unable to identify them as such. We would then expect to have complementary species for each protein family. An interesting example of this possibility would be the NUWM and NURM families since the first one is found only in Saccharomycotina species that have complex I and the other one is only found in Pezizomycotina species.

In the work published by Cardol et al. (2005), NUVM was proposed to be orthologous to the mammalian NDUFB4. In light of our analysis this seems unlikely, as NDUFB4 has other orthologs in fungal species, and not even with the high level of duplicates found within this subunit were we able to find any NUVM homolog. In fact, performing a blast search using the human NDUFB4 as a starting point produced few, non-reliable, hits in the fungal database (e-values >1.0), showing that the fungal NDUFB4 and NUVM are either not homologous to the mammal NDUFB4 gene or that they have diverged too much to be able to safely trace their evolutionary history back. Either way, the two genes do not show any overlap and so we believe that they are two different subunits of complex I.

15.3.2 Duplication of OXPHOS Genes

Duplications are important events that can have a great impact in the evolution of a given species. According to our data, the OXPHOS pathway has not been exempt of such process despite the predicted low incidence of duplications occurring in a protein complex. In our analysis (Marcet-Houben et al. 2009), we found that more than 75% of the proteins that are part of the OXPHOS pathway contain, at least, one duplication event. Table 15.2 summarizes the average number of copies per species found for each protein in the OXPHOS pathway. These values were calculated as the total number of homologs found for each gene (orthologs and paralogs) divided by the number of species that contained at least one copy of the given gene.

The gene balance hypothesis theorizes that genes that are part of a complex have a lower chance of retaining both gene duplicates (Papp et al. 2003). The reason for that is found in the change of stoichiometric relationships that would occur if the expression level of only one of the genes in a complex was suddenly doubled. Contrary to these expectations, we find that most genes have an average number of copies per species superior to 1.

Seven genes have an average number of copies per species higher than 2: NDUFB4 (complex I), SDHA (complex II), QCR1 and QCR2 (complex III), subunits alpha and beta (complex V), and the alternative NADH dehydrogenase.

Table 15.2 Table of subunits that form the oxidative phosphorylation pathway. Cells are colored according to the average number of copies per species of each subunit. This value is calculated as the number of homologs of the subunit contained in each fungal species divided with the number of species that have at least one copy of the subunit. Darker colors represent higher average number of copies and go from 1.0 to 4.0 by increments of 1.0

Average number of copies							
Complex I							
1.6.5.3	NAD1	NAD2	NAD3	NAD4	NAD4L	NAD5	NAD6
NDUFA1	NDUFA11	NDUFA12	NDUFA13	NDUFA2	NDUFA4	NDUFA5	NDUFA6
NDUFA8	NDUFA9	NDUFAB1	NDUFB3	NDUFB4	NDUFB7	NDUFB8	NDUFB9
NDUFS1	NDUFS2	NDUFS3	NDUFS4	NDUFS6	NDUFS7	NDUFS8	NDUFV1
NDUFV2	NI9M	NURM	NUVM	NUWM	NUXM	NUZM	–
Complex II				Alternative NADH dehydrogenase		Alternative oxidase	
SDHA	SDHB	SDHC	SDHD				
Complex III							
CytB	CytC	ISP	QCR1	QCR10	QCR2	QCR6	QCR7
QCR8	QCR9	–	–	–	–	–	–
Complex IV							
COX1	COX10	COX11	COX15	COX17	COX2	COX3	COX4
COX5	COX6	COX6A	COX6B	COX7	COX8		
Complex V							
SUB 8	SUB A	SUB α	SUB B	SUB β	SUB C	SUB D	SUB δ
SUB ε	SUB F	SUB G	SUB γ	SUB H	SUB J	SUB K	SUB OSCP

This represents that 8% of the genes in the OXPHOS pathway have an average number of copies higher than 2. For comparison, we scanned the *C. albicans* phylome, namely, the collection of phylogenetic trees for each gene in the *C. albicans* genome, in order to find genes that had an average number of copies per species greater than 2. The *C. albicans* phylome was constructed using 83 fungal species and can be found in phylomeDB (http://www.phylomedb.org) (Huerta-Cepas et al. 2010). Out of 5,824 genes, 951 had an average number of copies per species greater than 2, representing about 16% of the genes, which doubles the amount of genes found in OXPHOS. On the other hand, we find that 76% of the genes in OXPHOS have at least one duplication event, while in the *C. albicans* phylome 77% of the genes have at least one duplicate. This indicates that while the genes that are part of the OXPHOS pathway have been duplicated as often as all the other genes in the *C. albicans* genome, they tend to conserve less copies than other genes in the genome.

15.4 Examples of Evolutionary Events That Have Shaped the OXPHOS Pathway in Fungi

15.4.1 QCR1 and QCR2

Complex III subunits are mainly nuclearly encoded. This complex usually has four redox centers that are involved in electron transfer. There are two core proteins facing the matrix (QCR1 and QCR2) that show homology to mitochondrial peptidases involved in processing newly imported proteins. However the complex III subunits in yeast are proteolitically inactive and are not involved in cytochrome c reductase activity. Even so, the two proteins are needed for the correct assembly of the complex. These two proteins are homologous to the mitochondrial processing peptideases (Mas1 and Mas2 in yeast). They evolved from ancient duplication events that happened before the divergence between humans, animals, and plants. After this first round of duplications, no other duplications have occurred in these complex III subunits except for a species-specific duplication resulting from the WGD in *Rhizopus oryzae* (Ma et al. 2009).

15.4.2 ACPM Protein in Complex I

The ACPM protein found in complex I was predicted to be the only remaining protein that could be found in *Saccharomyces* species after the loss of complex I. The protein was found as part of the synthesis of octanoic acid and this change of function served as explanation for this group of species to have retained it. We showed that the functional change occurred before the loss of complex I in

Saccharomyces species and that it was related to a duplication event that occurred in Saccharomycotina species. The ACPM protein identified in yeast was actually the paralog to the original complex I protein. This was seen due to the fact that *Candida* species still retain both copies of the protein and a phylogenetic tree clearly showed that a duplication event had happened followed by a loss of one of the copies in yeast.

Yarrowia lipolytica also contains two copies of the protein. It was seen that both copies were associated to complex I (Dobrynin et al. 2010). Deletion of ACPM1, which is orthologous to the complex I subunit in *N. crassa*, was viable but the organism was unable to assemble complex I, which clearly depicts an association between complex I and ACPM1. On the other hand, deletion of the second copy, the ortholog to the yeast ACPM protein, was not viable, hindering the identification of its function. It is possible that ACPM2 would then be associated to the synthesis of octanoic acid and that its association to complex I is only used as a mechanism to be recruited to the membrane. In any case, further experimental proofs are needed to establish the functional differences between both paralogous groups.

15.5 Concluding Remarks

The OXPHOS pathway in fungi has undergone numerous changes during the evolution of this diverse group of species. Events such as the loss of complex I in yeasts adapted to fermentative lifestyles, the massive loss of the whole pathway in parasitic Microsporidia, and the large number of duplications detected in all the complexes of the OXPHOS pathway, have shaped the respiratory mechanisms of fungi and allowed this kingdom to expand over numerous different environmental niches. Additionally, the presence of alternative pathways to the primary electron transport chain may have been a turning point in many evolutionary events such as the shift from nonpathogenic to pathogenic or the shift of aerobic to anaerobic lifestyles.

The large number of duplications detected in the OXPHOS pathway belies the notion that proteins that are part of complexes are less likely to retain both copies after a duplication event. We saw how the percentage of proteins in OXPHOS that have, at least, one duplication was the same as the one found in the *C. albicans* genome. The only difference observed was that OXPHOS proteins tend to have less duplicates than other *C. albicans* genes, so there may be some restrictions acting on the retention of too many duplicates of the complex.

Acknowledgment TG and MMH are founded through a grant of the Spanish Ministry of Science (BFV2009-09168).

References

Abdrakhmanova A, Zickermann V, Bostina M, Radermacher M, Schagger H, Kerscher S, Brandt U (2004) Subunit composition of mitochondrial complex I from the yeast *Yarrowia lipolytica*. Biochim Biophys Acta 1658(1–2):148–156. doi:10.1016/j.bbabio.2004.04.019; S0005272804001446 [pii]

Anisimova M, Gascuel O (2006) Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. Syst Biol 55(4):539–552. doi:T808388N86673K61 [pii]; 10.1080/10635150600755453

Burri L, Williams BA, Bursac D, Lithgow T, Keeling PJ (2006) Microsporidian mitosomes retain elements of the general mitochondrial targeting system. Proc Natl Acad Sci USA 103 (43):15916–15920. doi:0604109103 [pii]; 10.1073/pnas.0604109103

Cardol P, Gonzalez-Halphen D, Reyes-Prieto A, Baurain D, Matagne RF, Remacle C (2005) The mitochondrial oxidative phosphorylation proteome of *Chlamydomonas reinhardtii* deduced from the Genome Sequencing Project. Plant Physiol 137(2):447–459. doi:137/2/447 [pii]; 10.1104/pp. 104.054148

Delsuc F, Brinkmann H, Philippe H (2005) Phylogenomics and the reconstruction of the tree of life. Nat Rev Genet 6(5):361–375. doi:nrg1603 [pii]; 10.1038/nrg1603

Dobrynin K, Abdrakhmanova A, Richers S, Hunte C, Kerscher S, Brandt U (2010) Characterization of two different acyl carrier proteins in complex I from *Yarrowia lipolytica*. Biochim Biophys Acta 1797(2):152–159. doi:S0005-2728(09)00263-1 [pii]; 10.1016/j.bbabio.2009.09.007

Duarte M, Peters M, Schulte U, Videira A (2003) The internal alternative NADH dehydrogenase of *Neurospora crassa* mitochondria. Biochem J 371(Pt 3):1005–1011. doi:10.1042/BJ20021374; BJ20021374 [pii]

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4):783–791

Fitzpatrick DA, Logue ME, Stajich JE, Butler G (2006) A fungal phylogeny based on 42 complete genomes derived from supertree and combined gene analysis. BMC Evol Biol 6:99. doi:1471-2148-6-99 [pii]; 10.1186/1471-2148-6-99

Gabaldon T, Huynen MA (2003) Reconstruction of the proto-mitochondrial metabolism. Science 301(5633):609. doi:10.1126/science.1085463; 301/5633/609 [pii]

Gabaldon T, Huynen MA (2004) Shaping the mitochondrial proteome. Biochim Biophys Acta 1659(2–3):212–220. doi:S0005-2728(04)00248-8 [pii]; 10.1016/j.bbabio.2004.07.011

Gabaldon T, Rainey D, Huynen MA (2005) Tracing the evolution of a large protein complex in the eukaryotes, NADH:ubiquinone oxidoreductase (complex I). J Mol Biol 348(4):857–870. doi:S0022-2836(05)00237-8 [pii]; 10.1016/j.jmb.2005.02.067

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52(5):696–704. doi:54QHX07WB5K5XCX4 [pii]

Hackstein JH, Tjaden J, Huynen M (2006) Mitochondria, hydrogenosomes and mitosomes: products of evolutionary tinkering! Curr Genet 50(4):225–245. doi:10.1007/s00294-006-0088-8

Hawksworth DL (1991) The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycol Res 95(4):641–655

Helmerhorst EJ, Murphy MP, Troxler RF, Oppenheim FG (2002) Characterization of the mitochondrial respiratory pathways in *Candida albicans*. Biochim Biophys Acta 1556(1):73–80. doi:S0005272802003080 [pii]

Huerta-Cepas J, Dopazo H, Dopazo J, Gabaldon T (2007) The human phylome. Genome Biol 8(6): R109. doi:gb-2007-8-6-r109 [pii]; 10.1186/gb-2007-8-6-r109

Huerta-Cepas J, Capella-Gutierrez S, Pryszcz LP, Denisov I, Kormes D, Marcet-Houben M, Gabaldon T (2010) PhylomeDB v3.0: an expanding repository of genome-wide collections of trees, alignments and phylogeny-based orthology and paralogy predictions. Nucleic Acids Res. doi:doi:gkq1109 [pii]; 10.1093/nar/gkq1109

Joseph-Horne T, Hollomon DW, Wood PM (2001) Fungal respiration: a fusion of standard and alternative components. Biochim Biophys Acta 1504(2–3):179–195. doi:S0005272800002516 [pii]

Keeling PJ, Corradi N, Morrison HG, Haag KL, Ebert D, Weiss LM, Akiyoshi DE, Tzipori S (2010) The reduced genome of the parasitic microsporidian *Enterocytozoon bieneusi* lacks genes for core carbon metabolism. Genome Biol Evol 2:304–309. doi:evq022 [pii]; 10.1093/gbe/evq022

Kerscher SJ (2000) Diversity and origin of alternative NADH:ubiquinone oxidoreductases. Biochim Biophys Acta 1459(2–3):274–283. doi:S0005-2728(00)00162-6 [pii]

Lavin JL, Oguiza JA, Ramirez L, Pisabarro AG (2008) Comparative genomics of the oxidative phosphorylation system in fungi. Fungal Genet Biol 45(9):1248–1256. doi:S1087-1845(08)00108-4 [pii]; 10.1016/j.fgb.2008.06.005

Letunic I, Bork P (2007) Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23(1):127–128. doi:btl529 [pii]; 10.1093/bioinformatics/btl529

Li Q, Bai Z, O'Donnell A, Harvey LM, Hoskisson PA, McNeil B (2010) Oxidative stress in fungal fermentation processes: the roles of alternative respiration. Biotechnol Lett. doi:10.1007/s10529-010-0471-x

Ma LJ, Ibrahim AS, Skory C, Grabherr MG, Burger G, Butler M, Elias M, Idnurm A, Lang BF, Sone T, Abe A, Calvo SE, Corrochano LM, Engels R, Fu J, Hansberg W, Kim JM, Kodira CD, Koehrsen MJ, Liu B, Miranda-Saavedra D, O'Leary S, Ortiz-Castellanos L, Poulter R, Rodriguez-Romero J, Ruiz-Herrera J, Shen YQ, Zeng Q, Galagan J, Birren BW, Cuomo CA, Wickes BL (2009) Genomic analysis of the basal lineage fungus *Rhizopus oryzae* reveals a whole-genome duplication. PLoS Genet 5(7):e1000549. doi:10.1371/journal.pgen.1000549

Magnani T, Soriani FM, Martins Vde P, Policarpo AC, Sorgi CA, Faccioli LH, Curti C, Uyemura SA (2008) Silencing of mitochondrial alternative oxidase gene of *Aspergillus fumigatus* enhances reactive oxygen species production and killing of the fungus by macrophages. J Bioenerg Biomembr 40(6):631–636. doi:10.1007/s10863-008-9191-5

Marcet-Houben M, Gabaldon T (2009) The tree versus the forest: the fungal tree of life and the topological diversity within the yeast phylome. PLoS ONE 4(2):e4357. doi:10.1371/journal.pone.0004357

Marcet-Houben M, Marceddu G, Gabaldon T (2009) Phylogenomics of the oxidative phosphorylation in fungi reveals extensive gene duplication followed by functional divergence. BMC Evol Biol 9:295. doi:1471-2148-9-295 [pii]; 10.1186/1471-2148-9-295

Marella M, Seo BB, Yagi T, Matsuno-Yagi A (2009) Parkinson's disease and mitochondrial complex I: a perspective on the Ndi1 therapy. J Bioenerg Biomembr 41(6):493–497. doi:10.1007/s10863-009-9249-z

Mueller G, Schmit J (2007) Fungal biodiversity: what do we know? What can we predict? Biodivers Conserv 16:1–5

Ohno S (1970) Evolution by gene duplication. Springer, New York

Papp B, Pal C, Hurst LD (2003) Dosage sensitivity and the evolution of gene families in yeast. Nature 424(6945):194–197. doi:10.1038/nature01771; nature01771 [pii]

Schmit J, Mueller G (2007) An estimate of the lower limit of global fungal diversity. Biodivers Conserv 16(1):99–111

Snel B, Huynen MA, Dutilh BE (2005) Genome trees and the nature of genome evolution. Annu Rev Microbiol 59:191–209. doi:10.1146/annurev.micro.59.030804.121233

Suvase SA, Annapure US, Singhal RS (2010) Gellan gum as an immobilization matrix for the production of cyclosporin A. J Microbiol Biotechnol 20(7):1086–1091. doi:JMB020-07-05 [pii]

Takaya N (2009) Response to hypoxia, reduction of electron acceptors, and subsequent survival by filamentous fungi. Biosci Biotechnol Biochem 73(1):1–8. doi:JST.JSTAGE/bbb/80487 [pii]

Visser W, Scheffers WA, Batenburg-van der Vegte WH, van Dijken JP (1990) Oxygen requirements of yeasts. Appl Environ Microbiol 56(12):3785–3792

Voncken F, Boxma B, Tjaden J, Akhmanova A, Huynen M, Verbeek F, Tielens AG, Haferkamp I, Neuhaus HE, Vogels G, Veenhuis M, Hackstein JH (2002) Multiple origins of hydrogenosomes: functional and phylogenetic evidence from the ADP/ATP carrier of the anaerobic chytrid *Neocallimastix* sp. Mol Microbiol 44(6):1441–1454. doi:2959 [pii]

Wang H, Xu Z, Gao L, Hao B (2009) A fungal phylogeny based on 82 complete genomes using the composition vector method. BMC Evol Biol 9:195. doi:1471-2148-9-195 [pii]; 10.1186/1471-2148-9-195

Williams BA, Elliot C, Burri L, Kido Y, Kita K, Moore AL, Keeling PJ (2010) A broad distribution of the alternative oxidase in microsporidian parasites. PLoS Pathog 6(2):e1000761. doi:10.1371/journal.ppat.1000761

Wittig I, Carrozzo R, Santorelli FM, Schagger H (2006) Supercomplexes and subcomplexes of mitochondrial oxidative phosphorylation. Biochim Biophys Acta 1757(9–10):1066–1072. doi:S0005-2728(06)00130-7 [pii]; 10.1016/j.bbabio.2006.05.006

Wolf YI, Rogozin IB, Grishin NV, Tatusov RL, Koonin EV (2001) Genome trees constructed using five different approaches suggest new major bacterial clades. BMC Evol Biol 1:8

Yagi T, Seo BB, Nakamaru-Ogiso E, Marella M, Barber-Singh J, Yamashita T, Matsuno-Yagi A (2006) Possibility of transkingdom gene therapy for complex I diseases. Biochim Biophys Acta 1757(5–6):708–714. doi:S0005-2728(06)00024-7 [pii]; 10.1016/j.bbabio.2006.01.011