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RECENT ADVANCES IN MYCOVIRUS RESEARCH*

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Introduction

Mycoviruses represent a structurally diverse group of viruses infecting fungi. Mycoviruses, like most plant viruses, do not have an extracellular phase in their multiplication cycle, and are transmitted only by intracellular routes (endogenous genetic elements) [1, 2]. Mycoviruses were discovered in the 1960s in *Agaricus bisporus* as possible causative agents of La France disease [3], and in *Penicillium* and *Aspergillus* species as interferon inducers, respectively [4]. They usually cause symptomless infections, although some of them are associated with one of the numerous phenotypic effects on the fungus. For example, die-back disease of *Agaricus*, killer phenomenon in yeasts, and hypovirulence in *Cryphonectria* parasitica are phenomena found to be associated with mycovirus infections (for reference, see [1, 2]). These properties of the mycoviruses are the driving forces of recent investigations.

Fungal prions have been first described in the 1990s in *Saccharomyces cerevisiae* [5] and later in *Podospora anserina* [6]. Recent data indicate that fungal prions can be used as models for examining the origin, structure and multiplication of mammalian prions causing fatal neuro-degenerative diseases like Creutzfeld-Jacob disease or bovine spongiform encephalopathy (BSE) [7].

In this review, we wish to give an overview of recent results in mycovirus and fungal prion research, with an emphasis on mycoviruses of plant pathogenic fungi, and on the evolution of viruses infecting fungi.

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Taxonomy and molecular organisation of mycoviruses

According to modern virus taxonomy, members of 8 virus families and of an unassigned genus infect fungi (Table I) [8]. Most mycoviruses carry dsRNA genomes enclosed into nonenveloped isometric virus-like particles (VLPs; Fig. 1). These viruses carry one or several dsRNA segments, and replicate by the action of an RNA dependent RNA polymerase (RDRP; Partitiviridae, Totiviridae, Chrysoviridae, Hypoviridae). Other mycoviruses resemble retrotransposons, and replicate by reverse transcription (Pseudoviridae, Metaviridae).

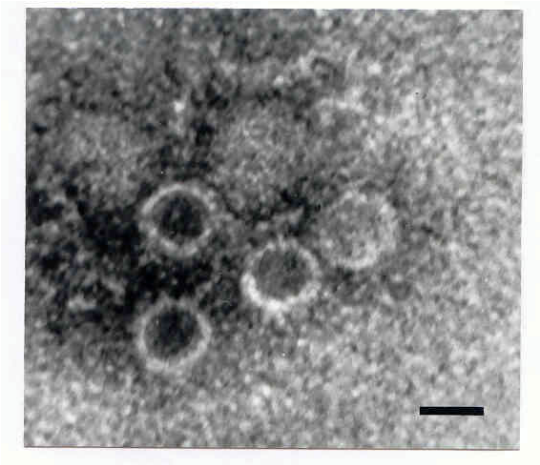

Fig. 1. Electron microscopic picture of isometric virus-like particles of about 35 nm in a *Mucor hiemalis* f. *corticolus* isolate. The bar represents 50 nm

Positive-strand ssRNA mycoviruses (Barnaviridae) and a dsDNA mycovirus infecting *Rhizidiomyces* species have also been described [8]. The latter species, however belongs to the *Hyphochytriomycota phylum*, which is not treated as member of the fungal kingdom [9]. Besides these viruses, a large number of mycoviruses are still uncharacterized. Rigid rod-shaped VLPs of rusts and powdery mildews; flexuous rods of *Boletus edulis* and other fungi; club-shaped VLPs of *Agaricus* species; Herpesvirus-like viruses of lower fungi; enveloped bacilliform particles of the entomopathogenic zygomycete *Strongwelsea magna*; numerous encapsidated and non-encapsidated dsRNA viruses of rusts; and bacteriophage-like VLPs of yeasts and *Penicillium* species have not yet been examined in detail [1, 10]. Interestingly, these latter viruses could be propagated in *Escherichia coli*, indicating their bacterial origin [1].

Table I

Taxonomy of viruses infecting fungi [8]

Nature of the genome	Family	Morphology	Genome structure	Genus	Type species	Closest relative (based on sequence data)
Ss(+)RNA	Barnaviridae	bacillliform	1 ss(+)RNA	Barnavirus	Mushroom bacilliform virus	Luteo-, carmoviruses
	Narnaviridae	RNP complex	1 + segment	Narnavirus	*Saccharomyces cerevisiae* 20SRNA narnavirus	Leviviridae
				Mitovirus	*Cryphonectria parasitica* mitovirus-1 NB631	Leviviridae, RDRPs of plant mtDNAs
DsRNA	Hypoviridae	pleomorphic	1 segment	Hypovirus	*Cryphonectria* hypovirus 1-EP713	Potyviridae
	Chrysoviridae	isometric	3 segments	Chrysovirus	*Penicillium chrysogenum* virus	Totiviridae
	Partitiviridae	isometric	2 segments	Partitivirus	*Atkinsonella hypoxylon* virus	Alphacryptovirus
	Totiviridae	isometric	1 segment	Totivirus	*Saccharomyces cerevisiae* L-A virus	Giardia-, Leishmaniaviruses, *Ribes,* Totivirus
Ss(+)RNA-RT	Pseudoviridae	spherical	1 + segment	Pseudovirus	*Saccharomyces cerevisiae* Ty1 virus	Hemivirus, Retroviridae, Metaviridae
				Hemivirus	*Drosophila melanogaster* copia virus*	Pseudovirus, Retroviridae, Metaviridae
	Metaviridae	spherical	1 + segment	Metavirus	*Saccharomyces cerevisiae* Ty3 virus	Caulimoviridae, Retroviridae, Pseudoviridae
DsDNA	-	isometric	1 circular	Rhizidiovirus	*Rhizidiomyces* virus	Phycodnaviridae

*includes *Saccharomyces cerevisiae* Ty5 virus

The genome sequence and organization of some mycoviruses have recently been characterized. Although most of these viruses could be assigned to one of the families listed in Table I, there are several exceptions (Table II). For example, a mycovirus (DaRV) of *Diaporthe ambigua* could not be assigned to any of the above families; its genome sequence exhibited relatively high levels of homology to carmoviruses of the Tombusviridae family (Table II) [11]. A dsRNA mycovirus found in *Fusarium graminearum* isolates resembles members of the Hypoviridae family and barley mosaic viruses [12]. A flexuous rod-shaped virus of *Botrytis cinerea* seems to be closely related to plant potex- and tymoviruses, while a *Cryphonectria parasitica* mycovirus carrying 11 dsRNA segments, and a *Rosellinia necatrix* virus which carries 12 dsRNA segments have been suggested to be members of the Reoviridae family [13–15]. The mycovirus responsible for La France disease of *Agaricus bisporus* [16] and a mycovirus of *Sclerophthora macrospora* [17] also remain unassigned. Features of these mycoviruses resemble those of virus families that did not include fungal viruses earlier, or represent new virus families. For a correct assignment, other characters of these viruses must also be examined (genome organization, morphology, mode of multiplication) before they can be grouped into a given virus family. Killer viruses of *Saccharomyces cerevisiae* and of other yeasts also could not be assigned to any virus families; these satellite viruses need a helper virus for replication and encapsidation,

since their genome lack an RDRP gene. Since most phylogenetic analyses use sequences of genes involved in replication of viruses (reverse transcriptases, RDRPs), it is not surprising that these killer viruses could not be classified properly.

Table II

Characteristics of recently identified unassigned mycoviruses

Host	Morphology	Genome	Closest relative	Reference
Diaporthe ambigua	RNA-RDRP complex	1 ssRNA	Tombusviridae	[11]
Botrytis cinerea	Rod-shaped	SsRNA with polyA	Tymo-, potex-like plant viruses	[13]
Agaricus bisporus	Isometric, 25 nm	2 dsRNAs	Totiviridae	[80]
Agaricus bisporus	Isometric, 34 nm	9 dsRNAs	?	[16]
Rosellinia necatrix	isometric	12 dsRNAs	Reoviridae	[15]
Fusarium graminearum	Pleomorph	1 dsRNA	Hypoviridae	[12]
Cryphonectria parasitica C-18	Isometric	11 dsRNAs	Reoviridae	[14]
Sclerophthora macrospora	Isometric	1 ssRNA	?	[17]

A phylogenetic tree of mycovirus families based on RDRP and reverse transcriptase sequences is shown in Figure 2. RNA viruses evolve at a much higher rate than those with a DNA genome due to the lack of proof-reading activities of RNA polymerases, according to the hypothesis of quasispecies (i.e. a single virus isolate is not a single sequence, but a swarm of mutant sequences that vary around a consensus sequence) [18]. Due to their high variability, such phylogenetic trees should be analyzed with caution. Nevertheless, most mycovirus families are well-separated on the tree. The recently characterized chrysoviruses of *Penicillium chrysogenum* and *Helminthosporium victoriae* are more closely related to the Totiviridae than to Partitiviridae supporting their assignment to a separate family, Chrysoviridae. Mitoviruses form a well-defined clade together with a putative mitochondrial RDRP gene of *Arabidopsis thaliana*, and are related to Narnaviruses [19]. Barnaviridae, and the unassigned viruses of *Botrytis*, *Agaricus* and *Diaporthe* species are more closely related to different plant or bacterial virus families than to any other fungal virus (Figure 2).

Occurrence of mycoviruses

Species of all phyla of the fungal kingdom can serve as a host for mycoviruses including Chytridiomycota, Zygomycota, Ascomycota and Basidiomycota. The first mycoviruses of zygomycetes were described in our laboratory [20–22]. Most research

has been done, however, on mycoviruses of ascomycetes and basidiomycetes. Regarding the occurrence of mycoviruses, the frequency of infection among isolates of a given species varies greatly. For example, all examined isolates of *Fusarium poae* were found to carry dsRNA segments indicative of mycovirus infection [23]. In *Aspergillus niger*, usually about 10% of the isolates are infected [24–26]. Similar frequencies were observed in other fungi including *Fusarium culmorum* and other *Aspergillus* species (Tóth, B., unpublished observations) [27]. In species where sexual reproduction is an important part of the life cycle in nature, infection rates are usually much lower (e.g. in *Fusarium graminearum*) [12]. In *Aspergillus nidulans*, none of the isolates examined sofar were infected with mycoviruses (Varga, J., unpublished observations) [28].

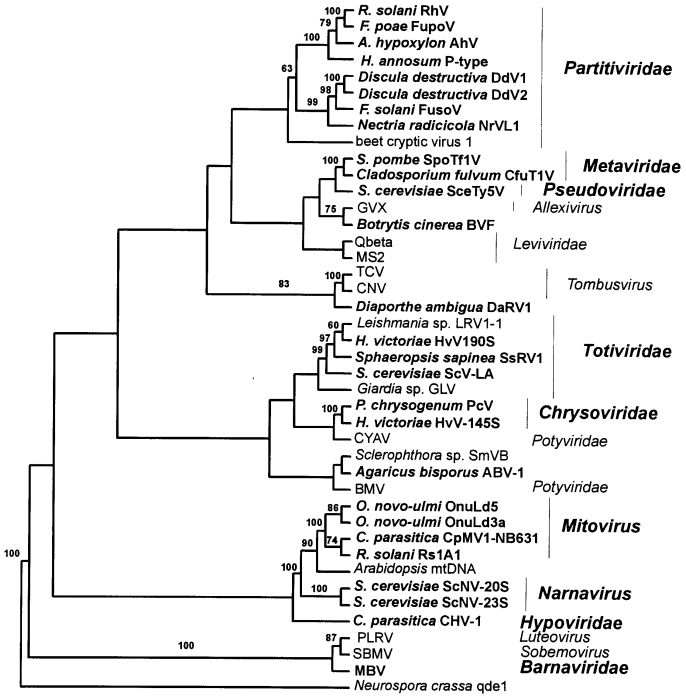

Fig. 2. Cladogram of mycoviruses based on conserved motifs A to E of RDRP and reverse transcriptase sequences. The sequences were obtained from the GenBank database. Alignments were carried out by the ClustalX program, while parsimony analyses were done by programs SEQBOOT, DNAPARS and CONSENSE of the PHYLIP software package [81]. Bootstrap values higher than 50 are indicated on the branches. A *Neurospora* RDRP gene (*qde1*) taking part in PTGS was used as outgroup. Abbreviations: BMV, barley yellow mosaic virus; CNV, cucurbit necrosis virus; CYAV, cucurbit yellows associated virus; GVX, garlic virus X; MBV, mushroom bacilliform virus; MS2, bacteriophage MS2; PLRV, potato leafroll virus; Qbeta, bacteriophage Qβ; SBVM, Southern bean mosaic virus; TCV, turnip crinkle virus

One fungal isolate can be infected by a number of different mycoviruses simultaneously. For instance, *Aspergillus foetidus* CBS 618.78 is infected with two viruses, AfV-S belonging to the Totiviridae, and AfV-F, an unassigned mycovirus giving rise to a 6-band pattern (Figure 3, lane 2). Observation of 1–8 different dsRNA segments in individual isolates of black Aspergilli indicates that even more mycoviruses can be present in a single isolate at the same time [26]. A single species can also be the host of various mycoviruses belonging to different virus families, e.g. different *Cryphonectria parasitica* isolates may carry viruses belonging to either Hypoviridae, Narnaviridae, or a virus resembling the Reoviridae family, or *Agaricus bisporus* may carry bacilliform ssRNA viruses (Barnaviridae), isometric viruses of the Partitiviridae family, either 25 nm or 34 nm isometric viruses carrying 2 and 9 dsRNAs, respectively (both are unassigned) [1, 14].

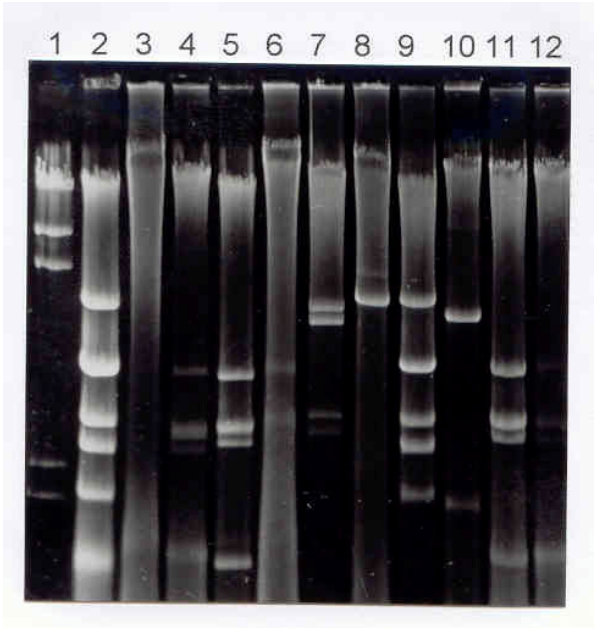

Fig. 3. Visualization of dsRNA elements of various fungal isolates on agarose gel. Lane 1, *Hin*dIII-digested lambda DNA; 2, *Aspergillus foetidus* CBS 618.78; 3–4, *Neosartorya quadricincta* isolates; 5–6, *N. hiratsukae* isolates; 7, *Micromucor ramannianus*; 8, *A. clavatus*; 9, *A. niger*; 10, *Saccharomyces cerevisiae*; 11, *N. hiratsukae*

Mycoviruses are usually located in the cytoplasm of the fungal host. Association of dsRNA molecules with the mitochondria was, however, detected in some fungi, including *Puccinia* species [29], *Saccharomyces cerevisiae* [30], *Cryphonectria*

parasitica [31], *Petromyces alliaceus* [27] and *Ophiostoma ulmi* [32]. Mycoviruses of *Rhizopus* isolates have also been detected in the mitochondrial fraction [22]. Some of these mycoviruses including those of *Cryphonectria parasitica, Rhizoctonia* and *Ophiostoma* species have been characterized in detail, and are assigned to the Mitovirus genus of the Narnaviridae family (Table I). RNA-dependent RNA polymerase genes of members of this family exhibit sequence relationships to putative RDRPs in the mitochondrial genomes of *Arabidopsis* and *Vicia* species [19, 33]. Interestingly, the presence of such dsRNAs in *Rhizoctonia solani* exalted virulence, while caused debilitation of the fungus, affected ceratoulmin production, and were suggested to be responsible for the *de novo* generation of mitochondrial DNA plasmids in *Ophiostoma* species [34].

Transmission of mycoviruses

Numerous attempts have been made to infect fungal mycelia with purified mycoviruses, usually without success [1]. However, *Saccharomyces cerevisiae* non-killer strains could be successfully transfected with purified VLPs, leading to the speculation that possibly this is the natural route of VLP infection of yeasts in nature [35]. Infection of protoplasts, transfer of mycoviruses through protoplast fusion or transfer of cDNA copies of viruses have been found to be useful tools for research purposes [1, 24, 36].

Mycoviruses can be vertically transmitted through asexual or sexual spores and hyphal fragments. Transmission through asexual spores is quite efficient in most cases (e.g. in black Aspergilli or *A. nidulans*) [26, 28]. In other species, transmission rates are much lower (e.g. in *Penicillium*, *Magnaporthe* species) [1]. For example, the 7 kb dsRNA fragment of *F. graminearum* was found to be transmitted to about 50% of conidia [12]. Transmission rates also varied in *Aspergillus flavus* isolates [37]. Transmission through ascospores is usually much less efficient; for example, a mycovirus transferred from *A. niger* to *A. nidulans* was found not to be transmitted into ascospores resulting from outcrossing [28]. Exclusion of dsRNA segments from sexual spores was also observed in *Gaeumannomyces graminis* strains [38], and in *Ophiostoma ulmi* [39]. On the contrary, mycovirus transfer through ascospores was found to be effective in *Neosartorya hiratsukae*, while the stromata embedding the asci in *Petromyces alliaceus* were found not to transmit one of the two observed dsRNA segments [27]. Mycoviruses are also transmitted very efficiently through basidiospores in *Agaricus brunnescens* [1], *Ustilago maydis* [40] and *Phaffia rhodozyma* [41], and through ascospores in *Saccharomyces cerevisiae* [42]. Transmission of mycoviruses to basidiospores in *Agrocybe aegerita* is, however, very weak [1].

Horizontal transmission of mycoviruses usually takes place through hyphal anastomosis. This process is under the control of vegetative or heterokaryon compatibility genes. In *Cryphonectria parasitica*, transmission occurs readily between isolates carrying the same *vic(het)* genes, while the efficiency of transmission decreases with increasing the number of different *vic* genes in the partners [43]. Similar findings were reported in *A. nidulans* [28].

Effect of mycoviruses on hosts

Killer phenotype

Killer strains of several yeast species secrete proteins toxic to sensitive cells of the same or closely related species. The producing cells are immune to this toxin. Most of the characterized killer toxins disturb membrane integrity [35]. These killer toxins and immunity are encoded on satellite dsRNAs in several yeasts (it can also be encoded on DNA plasmids or by nuclear genes in other species). The M1, M2 and M28 dsRNAs of *Saccharomyces cerevisiae* are encapsidated in virus particles, and need a helper virus (L-A virus in this species) for replication and encapsidation. DsRNAs with similar function have been found in other yeasts including *Hanseniaspora*, *Zygosaccharomyces, Phaffia* species, and also in *Ustilago maydis* [44]. Killer toxins may have several applications in medicine, food technology and agriculture (for details, see [35, 44]). The killer toxins of *Hanseniaspora* and *Zygosaccharomyces* species are especially promising as they have broad-spectrum antifungal activity against a number of human and plant pathogenic fungi including *Serpula*, *Heterobasidion*, *Fusarium*, *Candida* and *Sporothrix* species [35, 44].

Transmissible diseases

The presence of some mycoviruses results in disease symptoms including debilitated growth and poor spore germination in fungi. Such phenotypes have been observed in *Helminthosporium victoriae*, *Cryphonectria parasitica, Ophiostoma ulmi* (d^2 disease), and *Agaricus bisporus* (La France disease) [1]. In the former cases, mycovirus infected isolates have a potential for biological control of the diseases caused by the hosts (cereal foot rot, chestnut blight and Dutch elm disease, respectively), while in *Agaricus bisporus*, the mycovirus causes economical losses in mushroom growing areas [1].

Role of mycoviruses in plant pathogenicity

Several mycoviruses have been found to cause hypovirulence of the infected plant pathogenic fungus, e.g. mycoviruses of *Ophiostoma ulmi* [39], *Cryphonectria parasitica* [45], *Sclerotinia* species [46], *Diaporthe ambigua* [47], *Rosellinia necatrix* [15], and *Monosporascus cannonballicus* [48]. The presence of a 7.5 kb dsRNA element in *F. graminearum* was also found to correlate with decreased pathogenicity and lowered deoxynivalenol production [12]. On the contrary, mycoviruses of some fungi were found to upregulate fungal virulence leading to increased pathogenicity (e.g. dsRNA elements of *Nectria radicicola*, *Fusarium moniliforme* var. *subglutinans*, *Chalara elegans*) [49–52].

The mechanism of hypovirulence is best examined in *Cryphonectria parasitica*, where the hypovirus disturbes fungal development (sporulation and virulence) [45]. Virus infection reduces the levels of protein G in the cell, thus leading to enhanced cAMP accumulation [53]. At the same time, the virus also affects the secretion of proteins by using the vesicles of the secretory pathway for virus replication, resulting in decreased laccase, hydrophobin and cellulase secretion. These proteins are necessary for successful infection and invasion of chestnut trees. A possible explanation for the effect of this virus on host is silencing the homologous gene through post-transcriptional gene silencing (PTGS) [54, 55]. Gene silencing is due to sequence-specific degradation of mRNAs. Virus vectors containing inserts homologous to endogenous genes can induce PTGS. This mechanism was found to be widespread in plants and have also been observed in some fungi including *Neurospora* and *Fusarium* species [56, 57]. PTGS is thought to act as a defense mechanism against transposons and viruses. Although the mechanism of PTGS is largely unknown, there are some common features:

- PTGS can be induced either by transgenes or dsRNA molecules;
- PTGS is usually induced when a sequence homologous to the introduced element is also present in the genome;
- PTGS involves an RNA-dependent RNA polymerase both in plants and in fungi, and thought to be ubiquitous (PTGS involves small RNA molecules possibly synthesized by these RDRPs);
- PTGS is targeted mostly to the host mRNA and the virus itself is generally not eliminated.

In *Cryphonectria parasitica*, the hypovirus induced the same phenotype as a transgene homologous to a protein G α subunit indicating that PTGS might be involved

in the process [53]. The *Cryphonectria* hypovirus was successfully used to control chestnut blight in Europe, where the genetic variability of *C. parasitica* is low, but less successfully in the USA where this species displays huge diversity [45]. A cDNA copy of this hypovirus was successfully transferred to different lineages of *C. parasitica* as well as to related plant pathogenic species including other *Cryphonectria, Valsa* and *Phomopsis* isolates, where it induced the hypovirulence phenotype [58]. These experiments are promising as hypoviruses possibly could be used to control a wide range of plant pathogenic fungi. In *Fusarium graminearum*, the presence of a 7.5 kb hypovirus-related dsRNA virus correlated with altered growth rate, decreased mycotoxin production and decreased pathogenicity to cereals [12]. This mycovirus has a potential use in agriculture against *Fusarium* head blight, one of the most important fungal diseases of cereals like wheat and barley.

In *Rhizoctonia solani*, the presence of different dsRNA elements was found to lead to enhanced or decreased virulence, respectively [59, 60]. A virulence-associated dsRNA element (M1) was found to be a satellite virus with sequences homologous to a lactate-dehydrogenase enhancing virus of mice, which slows down the turnover of host serum enzymes, to plant bromoviruses and to a cytochrome c oxidase assembly factor. It was suggested that the virus upregulates the cytochrome oxidase complex thus speeding up ATP production leading to increased vigour [59]. Another, hypovirulence-associated dsRNA element (M2) was found to be related to mitoviruses. This virus exhibited homology to the pentafunctional AROM protein of the shikimate pathway, and its copies were also found on the chromosomal genome of the host [60]. Regarding the mechanism of hypovirulence, it was suggested that the virus reduces aromatic amino acid production through diverting intermediates of the shikimate pathway to the quinate pathway (according to the "pseudorepressor" hypothesis, the presence of M2 renders the quinate pathway constitutive) [61]. This leads to reduced production of phenylacetic acid, causing the same disease symptoms in plants as *Rhizoctonia* itself. As most of the characteristics of PTGS are present also in the M2 virus-*Rhizoctonia* system, the role of PTGS cannot be excluded in this interaction either.

Origin and evolution of mycoviruses

There are several independently evolving lineages of mycoviruses, and the different virus families including mycoviruses are clearly of different origin (Fig. 1, Table I) [1, 2]. Buck stated that most "(myco)viruses evolved at a very early stage in the phylogeny of their hosts" [14]. RNA viruses may have originated from the pre-DNA era, or more probably, from mRNAs of introns, exons or transposable elements. It was also suggested that viruses probably emerged from the sea with their different

hosts during the successive waves of colonisation of the land. Later on, viruses co-evolved with their hosts giving rise to their enormous diversity observed today in general, and in those infecting fungi in particular [2, 14]. Most mycoviruses utilize RDRP for replication. This enzyme lacks proof-reading activity resulting in an accelerated evolution of mycoviruses relative to their hosts. This "coevolution" hypothesis is supported by several observations including the following ones [1, 2, 14]:

- about 30 chromosomal genes are needed for replication and expression of killer viruses of *Saccharomyces cerevisiae*;
- mycoviruses located in the mitochondria of fungi adapted to their environment by using the mitochondrial genetic code for translation;
- a mycovirus (Hv190) of *Helminthosporium victoriae* utilizes host-encoded enzymes for processing its capsid protein.

However, several features of mycoviruses cannot be explained by the above hypothesis. If coevolution is assumed, sequence divergence of fungal viruses should presumably reflect divergence of their fungal hosts, i.e. the trees of hosts and parasites should be congruent [62]. The evolutionary trees of dsRNA viruses and their hosts are in fact incongruent; e.g. fungi are most closely related to animals on host trees, while most fungal dsRNA viruses have relatives among viruses infecting plants (see Table I, Fig. 1). Besides the relationships listed in Table I and depicted in Fig. 2, a plant virus isolated from *Ribes* cultivars has also been found to be closely related to the Totiviridae family [63]. Additionally, phylogenetic analyses suggest a relatively close relationship between Chrysoviridae and a chloroplast associated dsRNA virus of *Bryopsis* sp. (Genbank accession number: AB070653; data not shown), while RDRP sequence of the L2 dsRNA element of *Rhizoctonia solani* is related to dsRNA elements of rice and *Vicia faba* [61]. These observations suggest that horizontal gene transfer played an important role in mycovirus evolution, so some mycoviruses might be descendants of acquired plant viruses (although the alternative scenarios that either parallel evolution or transfer of fungal viruses to plants is responsible for the observed phylogenetic relationships cannot be rejected either). Recently, transfer of plant DNA sequences to *A. niger* [64] and nucleic acid transfers between fungal viruses and plants have also been reported [33], supporting the latter hypothesis. Such horizontal gene transfers are widespread in bacteria where cytoplasmic elements like plasmids encoding different traits are readily transferred to other species [65]. Several fungi and fungus-like organisms act as vectors of plant viruses in soil [66]. For example, chytrids like *Olpidium brassicae* can spread plant viruses belonging to several genera including Tombusvirus, Carmovirus, Necrovirus and Dianthovirus [66]. Higher fungi belonging

to the Basidiomycota and Zygomycota phyla were also suggested to be able to transmit plant viruses [67, 68]. Although the plant viruses are transmitted *in vitro* by these organisms (the viruses are attached to the surface of fungal thalli, but do not enter the cytoplasm), they could possibly be acquired accidentally.

Other mycoviruses belonging to Narnaviridae and Barnaviridae families resemble bacterial viruses, while Mitoviruses are associated with mitochondria that are of bacterial origin according to the endosymbiont hypothesis. The observation that some mycoviruses can be propagated in bacteria, and that gene sequences can be transferred between bacteria and fungi indicate that these mycoviruses are descendants of bacterial viruses [1, 65].

The close relationship of mycoviruses infecting phylogenetically widely separated fungal species indicates that mycoviruses might have also been transferred between different fungal species during evolution. For example, mitoviruses have been observed in two ascomycete and in one basidiomycete fungus; similarly, Partitiviruses have been detected both in ascomycetes (e.g. *Fusarium*, *Atkinsonella* and *Nectria* species), and in basidiomycetes (e.g. *Rhizoctonia, Heterobasidion* species) [8]. The observations that dsRNA elements could be successfully transferred from *A. niger* to *A. ficuum*, *A. oryzae* and *A. nidulans*, and also from *Fusarium poae* to *A. niger* isolates support this idea [28, 69, 70]. Such interspecific transfers could explain the close relationship of some mycoviruses infecting widely separated fungal taxa. Moreover, mycoviruses also acquired gene fragments from the hosts, e.g. *Rhizoctonia solani* M2 virus is related to a protein of the shikimate pathway, while M1 is phylogenetically related to a cytochrome c oxidase assembly factor [61]. Such “captured” genes have been found to be widespread among DNA viruses like Poxviridae and Herpesviridae [71].

The above observations indicate that at least some mycoviruses originated from plant viruses, while some others came from bacteria some time in the past. During adaptation to the new host, a major shift in the swarm of the quasispecies would be predicted due to dramatic environmental changes [18]. Unnecessary sequences (e.g. those required for cell-to-cell movement) were lost, and new sequences could be gained in some cases from the host. Finally, coevolution with the host together with the events mentioned above could have resulted in the present-day diversity of mycoviruses. However, further studies are necessary to get clearer insight into the evolution of mycoviruses.

Fungal prions

Although not mycoviruses, fungal prions should also be mentioned in this review. Prions are protein-based infectious particles that lack nucleic acids [72]. These elements are produced as a result of aberrant folding of a cellular protein into an infectious form. In yeasts, heritable elements exhibiting aberrant properties have been described since the 1960s [73, 74]. These elements produce dominant phenotypic traits, heritable in a non-Mendelian manner and transmissible by cytoduction. These phenotypes are [PSI+] and [URE3]. Prions have been first suggested to be implicated in these phenotypes in the 1990s in *Saccharomyces cerevisiae* [5] and later in *Podospora anserina* [6]. Fungal prions differ from mammalian prions in a number of ways including: (i) reversible curability; (ii) overexpression of the cellular protein leads to the formation of more prions; (iii) the prion phenotype is identical with the phenotype of a mutation in the corresponding cellular gene; (iv) prion formation is dependent on the presence of an active chaperon (Hsp104) [75]. In accordance with the divergent features of mammalian and fungal prions, even a new name ("propagons") has been proposed recently for prions of fungi [76]. All fungal prions form amyloid fibers in the fungal cell, that are reminiscent to those associated with Alzheimer's and Huntington's diseases. For propagation, all fungal prions require the presence of an active *Hsp104* protein, that is a chaperon affecting protein folding. Deletion of the *Hsp104* gene results in irreversible eradication of prions [7]. Four prions have been unequivocally identified in fungi, although it is suspected that other fungal traits might also be caused by prions. Three prions of *Saccharomyces cerevisiae*, [URE3], [PSI+] and [RNQ1] ([PIN+]) are altered infectious forms of the functional proteins *Ure2p*, *Sup35p* and *Rnq1p*, respectively [76]. The N-terminal parts of these proteins (the "prion domains") are responsible for prion formation and propagation and are rich in asparagine and glutamine residues. [URE3] makes cells able to take up ureidosuccinate or allantoate from the environment even in the presence of ammonia or other good nitrogen sources through releasing nitrogen catabolite repression caused by the *Ure2* protein, while [PSI+] decreases the efficiency of translation termination for which the *Sup35* protein is needed. These prions interact with each other; both [RNQ1] and [URE3] can promote the generation of [PSI+] [77]. The [PSI+] prion has strong effect on colony morphology and growth rate [7]. The normal form of this prion participates in translation termination, thus the prion form may promote the generation of new protein products through reducing the fidelity of protein synthesis, resulting in increased phenotypic diversity. Accordingly, this prion was suggested to be useful from an evolutionary point of view [77]. The only known prion in filamentous fungi is the [Het-s] prion of *Podospora anserina*. This prion is necessary to a normal cellular function, the heterokaryon incompatibility. [Het-s] has also been suggested to be

responsible for the "spore-killing" phenomenon observed in some natural *Podospora anserina* isolates [78]. The het-s protein has no evident similarity to other putative prion proteins. Other prion candidates include [GR] (provides resistance to glucosamine in *S. cerevisiae*), [KIL-d] (regulates expression of killer virus proteins in *S. cerevisiae*), and [C+] (causes crippled growth in *Podospora anserina*)[7]. Several other morphological variations observed in filamentous fungi might also be caused by prions [79].

In conclusion, recent advances in mycovirus and fungal prion research gave insight into the organization and function of these elements. Our knowledge on these elements is however still lagging behind that of plant or animal viruses or mammalian prions. Further investigations are necessary to clarify the evolutionary origin and significance of fungal viruses and prions. We hope that our actual studies on *Aspergillus*, *Fusarium* and zygomycete mycoviruses will contribute to the understanding of some features of these elements.

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