Fungal Viruses

Edited by
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Including Abstracts of Papers of the Symposium on
Extrachromosomal Vectors in Fungi and
Abstracts of Posters on Fungal Viruses

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With 78 Figures

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Preface

This book records the contributions presented at the XIIth International Congress of Microbiology, Mycology Section, held in Munich on 3–8 September 1978. All the papers given at the Symposium (no. 33) on Fungal Viruses, and at the Round Table Discussion (RTD 1) are reported in full, and the paper on fungi as vectors of plant viruses by R.N. Campbell (who was unfortunately unable to attend the Congress) has also been included (Part A).

Much of the current work with viruses in fungi involves genetic studies and virus-host gene interactions; for this reason, the Symposium (no. 32) on Extrachromosomal Vectors in fungi has also been reported, in the form of abstracts from all those contributors who gave permission for this (Part B).

Authors' abstracts of posters relating to fungal viruses have similarly been given (Part C).

Fungal viruses, or mycoviruses, can be defined as viruses that replicate in fungi, and since their discovery in 1962, considerable progress has been made towards an understanding of their biological and particularly their physico-chemical properties. Present knowledge suggests that mycoviruses are usually latent in nature, and their biological manifestations can often be more readily studied as cytoplasmically inherited determinants than as viruses. An attempt has therefore been made in this book to bring together the most recent advances, not only in mycovirus research, but also in extrachromosomal determinants in fungi.

Mycoviruses have been found in fungi from all the major taxonomic groups. Most of these viruses have been described only by electron microscopy, and they are therefore usually referred to as virus-like particles (VLP). During the last few years, however, purification procedures have been applied to more of these viruses, and many have now been characterized *in vitro* sufficiently to indicate their typical virus nature: these are generally referred to as mycoviruses. The general lack of transmission techniques has largely precluded the demonstration of infectivity of mycoviruses.

Although mycoviruses have been studied in only a limited number of laboratories around the world, it has become evident that these viruses are very common; the great majority of those examined have

been shown to contain a double-stranded ribonucleic acid (dsRNA) genome, and they thus represent the largest recognized group of dsRNA viruses. Taxonomically and biologically, however, they appear to differ considerably from the dsRNA viruses of the family Reoviridae. Whereas viruses in the Reoviridae are considered to be disease agents, there is very little adequately controlled evidence as to whether most mycoviruses are truly latent, or whether they induce more subtle cytopathic effects in their hosts. Mycoviruses appear to be intimately associated with their hosts and in some instances they control or modify certain biological attributes of the host cell; as such, they are an important part of mycology.

In the early days of mycovirus research, several topics were investigated because of their immediate practical application in the fields of pharmacology and medicine. The possible influence of mycoviruses on antibiotic production in *Penicillium* species and other industrial fungi, and on the production of mycotoxins by spoilage fungi of food products, received much attention. In both these important aspects, however, mycoviruses appeared to exert no significant effect. The potential clinical or veterinary uses of the dsRNA of mycoviruses as an interferon-inducing agent were explored, but the toxic side-effects soon discouraged further investigation. More recently, the possibilities have been examined of using mycoviruses that infect plant-pathogenic fungi as a means of biological control of these fungi. So far, there has been no consistent evidence that the observed hypovirulence in these fungi is actually caused by mycovirus infection, and this problem is further complicated by the difficulties in experimental transmission of the viruses concerned.

This lack of suitable techniques for experimental transmission remains the most serious limitation to mycovirus research today, and it is therefore all the more remarkable that so much information on the replicative strategies of different mycoviruses within the host cell has been elucidated, and that the complexities of the killer systems in *Saccharomyces* and *Helminthosporium* species have been at least partly unravelled. The killer systems have provided a unique experimental tool for studying the interactions of determinants carried in the mycovirus genome and those carried in the fungal chromosomes and cytoplasm.

Besides the mycoviruses, which infect fungi, there are a number of plant viruses that are transmitted by Chytrid fungi, although there is no evidence that any of these plant viruses can multiply in its fungal vector. The subject of fungi as vectors of plant viruses is included in the scope of this book.

The full biological significance of mycoviruses to their hosts can not yet be evaluated, but their very prevalence and efficient perpetuation ensure that they cannot be ignored by any who study fungi. This book represents an overview of the present state of our knowledge.

Thanks are due to the many people whose willing help and cooperation have made possible the production of this book. The generous financial assistance of the Deutsche Forschungsgemeinschaft, Bonn, is acknowledged; this not only made it possible for a number of overseas speakers to attend the symposium in Munich, but also enabled H.P.M. to attend the origanisational meeting during the 2nd International Mycological Congress in Tampa, Florida.

Finally, it is a pleasure to thank Miss Eva Grajf for her careful help in typing and proofreading and the editorial staff of Springer-Verlag for their kind cooperation and expert assistance.

October, 1979

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Contents

Part A

Symposium and Round Table Discussion on Fungal Viruses

Chairmen: M. HOLLINGS and H. A. WOOD
Convenor: H. P. MOLITORIS

Evolution

Coevolution of Fungi and Their Viruses

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1 Introduction

Evolution is a safe subject for discussion, as one can afford to speculate without cause for contradiction. A discussion on the coevolution of fungi and their viruses will be especially speculative, as it is based on the biological attributes of present-day fungi and on the properties of rather few viruses, those double-stranded RNA (dsRNA)-containing mycoviruses that have been physicochemically well characterized.

2 Virus-Host Coevolution

The physicochemical properties of dsRNA mycoviruses relative to dsRNA viruses of nonfungal hosts have been compared previously by Wood (1973). The biological properties of the dsRNA mycoviruses have been discussed extensively by others (Bozarth, 1972; Lemke and Nash, 1974; Lemke, 1976a; Hollings, 1978; Saksena and Lemke, 1978) and indicate that most if not all mycoviruses are heritable viruses – endogenous to the host cell not only during replication but during transmission. Their transmission occurs either during cell division (serial transmission) or following cell fusion (lateral transmission). Serial transmission is accomplished effectively in those fungi producing spores such as conidiospores (mitotic spores). Indeed many fungi, sexual as well as asexual fungi, produce such spores in great profusion. Other fungi produce spores of different types such as basidiospores (meiotic spores) or arthrospores (mitotic spores produced by fragmentation of vegetative cells). Regardless of spore type, all fungal spores, if they incorporate and retain virus particles in situ, are potential agents for serial transmission. Lateral transmission accompanies plasmogamy between genetically compatible fungi either during mating of sexually compatible strains or during fusion between vegetatively compatible strains regardless of their potential for sexual reproduction. Vegetative incompatibility, either within the species or between species, promotes inbreeding, and such inbreeding would delimit lateral transmission of mycoviruses. It is indeed interesting to speculate in this context that mycoviruses, as heritable agents, could well have afforded the selective pressure for inbreeding and perhaps even loss of sexual competence among fungi. Inbreeding mechanisms are common in fungi and have been discussed previously by the author (Lemke, 1973) and in textbooks devoted to fungal genetics (Esser and Kuenen, 1967; Burnett, 1975). There are thus far no adequate models to explain the selective advantage for inbreeding and apomixis in fungi.

The endogenous and noninfectious nature of mycoviruses characterized to date presupposes a compromise – an infection that is both latent and persistent. Latency benefits the host for survival, and persistence benefits the virus in the absence of infectivity. Clearly this compromise, while not typical of virus-host relationships, might well have evolved in response to an opportunity for more efficient viral transmission, lateral as well as serial, when integrity of a host system is maintained. Fungal systems may well have afforded this opportunity by their characteristic cellular organization, their potential for cytoplasmic exchange between vegetative cells, and their efficient reproduction through spores.

Host-mediated transmission, however, might lead to considerable loss of integrity in organization of a virus, and this indeed seems evident from physicochemical details on mycoviruses (Wood, 1973; Lemke, 1976a). The dsRNA mycoviruses, unlike other dsRNA-containing viruses, are frequently multicomponent (i.e., genome segments are distributed among a population of particles rather than in a single particle). Supernumerary molecular weight forms of dsRNA, including dsRNA molecules nonessential for production of virus particles, exist for some fungal viruses (Vodkin et al., 1974; Adler et al., 1976; Koltin, 1977; Lemke, 1977). Data on sedimentation of viral components indicate that large numbers of particles may be empty, and in one fungal system, *Aspergillus flavus* (Wood et al., 1974), all particles assembled are devoid of nucleic acid. These latter particles truly deserve to be called "viruslike particles."

2.1 Virus Concept

Since the fungal viruses characterized to date do not satisfy well all criteria expected of a virus, many investigators have adopted the term "viruslike particles" or "VLP" for these particles. Use of VLP, however, simply avoids the issue as to the nature of such particles. The term "defective" viruses has been considered, but this term implies degeneracy and is unfortunate, since the viruses of fungi, although relatively simple in structure, may be highly evolved and well-adapted to their host. A case for this will be presented below. In this paper, the term "heritable" viruses is preferred as rather descriptive of fungal viruses, which have thus far proven to be infectious only through heredity. Ultimately, fungal viruses may be found that are routinely infectious, and such viruses would of course not fit this description.

2.2 Virus Evolution as Evidenced by Reduction

Evolution from more complex progenitors to simpler forms, through reduction or loss in structure and function, has occurred repeatedly in the evolution of life forms, and for higher organisms this is often documented in the fossil record. Evidence for evolution by reduction of fungal viruses is indirect but can be gleaned from the comparative physicochemical properties of several fungal virus systems. For example, two very closely related fungi, *Penicillium cyaneofulvum* and *Penicillium chrysogenum*, each contains serologically identical viruses for which the capsid proteins and amino acid composition are identical (Buck and Girvan, 1977). These two viruses, however, differ

in genome complexity, as the virus of *Penicillium cyaneofulvum* contains an extra segment of dsRNA. Extra and apparently superfluous dsRNA segments are found also in the viruses of *Saccharomyces cerevisiae* (Vodkin et al., 1974; Adler et al., 1976) and *Ustilago maydis* (Koltin, 1977) isolated from specific host strains. Indeed, determinants for phenotypes other than virus structure and replication such as the toxin and immunity determinants in the killer systems of these fungi reside in such extra segments. In the light of the theory for evolution by reduction, these killer systems might well be regarded as dynamic systems in which such an evolution is still in progress.

It has already been suggested in this paper that an endogenous or heritable virus, in addition to loss of infectivity, could attain considerable reduction and modification in structure. The *Penicillium stoloniferum* slow virus, for example, which has been well characterized both for structure and replication (Buck and Kempson-Jones, 1973, 1974; Buck, 1975; and see the chapter by Buck in this volume) is surely to be considered a simple virus, although there is no direct evidence to prove that its simplicity evolved by reduction. Regardless, this virus has only two genome segments – one sufficiently large to encode for the single major capsid polypeptide and one sufficiently large to encode for the viral specific replicase. There are no excesses. This heritable virus is a model for adaptation for endogenous replication; it retains a minimal genome for maintenance of what might still qualify as a virus (i.e., a nucleoprotein particle able to replicate in a host cell). Further reduction might lead to a lone genome segment able to encode for its replicase. This has not been identified in fungal cells, but such a determinant, admittedly nonviral in organization, is conceivable as the ultimate product of viral evolution via reduction.

With reference to fungal viruses, three points deserve to be mentioned here. First, the spectrum of dsRNA mycoviruses indicates considerable heterogeneity for these viruses both in serology and genome segmentation (Wood, 1973; Lemke, 1976a; and see chapter by Bozarth in this volume). Thus, if reduction has occurred in the evolution of fungal viruses, it has apparently occurred repeatedly from several lines of viral progenitors. Secondly, in view of the prevalence of dsRNA-containing viruses in fungi, it would appear that the dsRNA genome may be preferentially suited for reduction leading to endogenous and persistent infection characteristic of a heritable virus. Indeed, models for replication of dsRNA fungal viruses indicate replicative cycles that are abbreviated when compared with the replication of dsRNA viruses from nonfungal hosts. The replication of fungal viruses, as heritable and persistent viruses, may well have been modified to keep pace with but not to exceed the rate of host cell division.

2.3 Host Evolution as Evidenced by Nuclear Gene Mutations

Indicative evidence that fungi and their viruses have coevolved and are coadapted to a considerable degree rests on the latent or symptomless relationship of most fungal viruses with their hosts. Specific evidence for coadaptation has been obtained from genetic studies with the yeast *Saccharomyces cerevisiae* (Wickner, 1974, 1978; Wickner and Leibowitz, 1976) and the mold *Penicillium chrysogenum* (Lemke et al., 1976). In the yeast there are more than 20 nuclear genes required for maintenance of the dsRNA segment encoding for determination of killer phenotype. This extra dsRNA segment,

specifically, and not the virus of yeast is maintained by the wild-type or dominant alleles of these nuclear genes; mutation leads to loss of this extra dsRNA. This latter point is significant in view of the already discussed potential for reduction in the evolution of fungal viruses. The studies with *Penicillium chrysogenum* indicate that the dominant allele of at least one nuclear gene is required for maintenance of the host when virus titer is high (Lemke et al., 1976). In the wild-type *Penicillium chrysogenum* the association of virus and host is symptomless as in most fungal viruses. Only a mutant strain of *Penicillium chrysogenum,* grown on a lactose-containing medium to enrich for virus titer, showed tissue degeneration or conditional lysis. The general absence of lysis or of disease symptoms in fungi containing viruses may be related not only to evolutionary reduction of these viruses but to evolution of host genes to accommodate endogenous viral infection. The absence of such evolution on the part of the host could lead to a pathogenic response to viruses, as is observed in the case of the mushroom virus disease of *Agaricus bisporus* (Hollings, 1962; Dieleman-van Zaayen, 1972; Lemke, 1976b).

2.4 Gene Frequencies and Heritable Viruses

The only mathematical models for evolution (change in gene frequency) of heritable viruses are based on analysis of certain viruses, principally the sigma virus, in populations of *Drosophila* (L'Héritier, 1970). Basically, three conclusions were drawn from this study, and these are mentioned here only because of their possible relevance to the study of fungal viruses. First, if inheritance of a virus is strictly maternal but incomplete (i.e., the virus is never transmitted through the sperm and is occasionally absent from the egg), then a totally noninfectious virus in order to be retained in a breeding population must confer some selective advantage upon the host. Secondly, if transmission is bidirectional and efficient (i.e., either parent may transmit the virus or, as in the case of many fungi, where cytoplasmic exchange is not limited to gametes and is possible through vegetative cell fusion), then a heritable virus may persist and indeed spread even without a selective advantage. By extrapolation, this second set of circumstances may well reflect what is common for mycoviruses, viral latency yet persistence without the aid of natural selection. Thirdly, heritable viruses, if they are detrimental or pathogenic to the host, and indeed noninfectious, should be lost readily from a breeding population, especially if the viral determinants are extrachromosomal and not linked to essential genetic elements. Of special relevance in this context, and seemingly contradictory, is the mushroom virus disease of *Agaricus bisporus,* a disease that is correlated with the presence of double-stranded RNA and with several morphological types of virus (Hollings, 1962; Dieleman-van Zaayen, 1972; Saksena, 1975; Marino et al., 1976; Lemke, 1976b; Del Vecchio et al., 1977, 1978). This disease involves heritable viruses that are pathogenic yet persistent and widespread among cultivated strains of *Agaricus bisporus.* It might be suggested either that heritable determinants for this disease are linked to essential genetic factors and are thereby retained (i.e., perhaps integrated DNA copies of these viruses exist in *Agaricus bisporus*) or that mushroom viruses, like other fungal viruses, are basically latent but are allowed to reach unnatural or pathogenic levels during the process of mushroom cultivation. Further

study of mushroom virus disease should focus upon such alternatives. The infectivity of mushroom viruses as free particles, considered to be negligible to absent on the basis of early work, should also now be reinvestigated in the light of more sensitive assays for virus detection (see chapters by Del Vecchio et al. and Lister in this volume). If mushroom viruses are indeed infectious, then the contradiction alluded to above does not exist.

3 Concluding Remarks

This essay depicts the known fungal viruses as heritable elements that have evolved by reduction in structure and function, as well as in potential for infectivity, from an extended series of more complex viruses. It is doubtful that details in this evolution can ever be reconstructed, but the serological and physicochemical data available to date indicate that this evolution occurred repeatedly among unrelated viruses and selectively among viruses with double-stranded RNA genomes. These events, although not retraceable, have led investigators of fungal viruses to reevaluate those criteria normally attributed to a virus. The fungal viruses, with perhaps one or two known exceptions, do not represent pathogenic or infectious agents; they are in concert with their host. Such associations could only have arisen through coevolution of fungi and their viruses, and each association should be studied seriously if we are to develop a classification for fungal viruses that is both meaningful and natural.

Summary

Fungal viruses are principally dsRNA-containing and endogenous to their host. Most, if not all, are transmitted laterally by cytoplasmic exchange or serially through spores or other propagules. Since they are infectious (lateral transmission) by cytoplasmic heredity, their spread is defined by incompatibility genes, especially genes for vegetative incompatibility. These viruses, despite similarity in appearance and simple structure, are heterogeneous for genome segmentation and serological relationships. Such viruses may be simple by reduction and represent residual forms of more complex progenitors. Their endogenous nature implies a close interaction with host genes for maintenance and replication, as well as lateral transmission. Fungal viruses offer model systems for study of nucleo-cytoplasmic interactions in eukaryotic cells. Any evidence for informational DNA copies of dsRNA segments would be most exciting, since this implies reverse transcriptase activity and the possibility of vector relationships involving recombinant DNA.

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Fungi as Vectors and Hosts of Viruses

Fungal Vectors of Plant Viruses

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1 Introduction

This paper is concerned with plant viruses (or viruslike agents) that are soil-borne and particularly with those having a fungal vector. These viruses are intimately associated with the fungus, often being harbored within the fungal resting spore. Nevertheless, they do not seem to multiply within the fungus. The emphasis in this paper will be on the fungus–virus relationships that are of biological and epidemiological interest.

2 General Characteristics of Fungal Transmission

2.1 Definitions of Soil-Borne and Soil-Transmitted Viruses

In this paper "viruses" will mean plant viruses and viruslike agents that cause plant diseases. A distinction will be made between "soil-borne" viruses and "soil-transmitted" viruses. The broader term "soil-borne" will denote a virus that is introduced into the soil and that subsequently infects healthy plants. The virus can be introduced into soil by incorporation of infected plant material, by release from the roots of living plants, by experimental addition to the soil as a purified or partially purified preparation, or by a soil-borne vector. All plant viruses enter the soil with infected host tissue. For most, however, this constitutes a "dead-end" as they have no effective means of surviving or being inoculated into succeeding host plants. Tobacco mosaic virus, to be discussed later, is the prime example of a virus adapted to this method. Experimentally it is also possible to add relatively large amounts of virus to the soil in the form of purified or partially purified preparations and to demonstrate infection of susceptible plants. Presumably infection occurs because the virus has saturated the root surface and there is mechanical damage to cells of the root system. This kind of damage is possible in spite of attempts to avoid it if the pots are moved about or even if only the tops of plants are moved resulting in physical abrasion or damage to root cells, or if there is injury from the soil fauna. Growth of the root tip or root hairs in soil does not by itself result in injuries that can serve as infection sites. A comparable type of soil-borne infection occurs when susceptible plants are removed from pots and new plants are transplanted into the same soil. In this case the mechanical transmission to the roots is obvious. While the virus in either case can be called soil-borne, this hardly constitutes evidence that the virus is soil-borne in nature or that the virus should be expected to have a soil-borne vector. The term "soil-transmitted" will be used when there is clear evi-