VIRUSES AND PLASMIDS IN FUNGI

Edited by
PAUL A. LEMKE

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SERIES ON MYCOLOGY

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Volume 1 Viruses and Plasmids in Fungi,
edited by Paul A. Lemke

Volume 2 The Fungal Community: Its Organization and Role in the Ecosystem,
(in preparation), *edited by Donald L. Wicklow and George C. Carroll*

INTRODUCTION TO THE SERIES

Mycology is the study of fungi, that vast assemblage of microorganisms which includes such things as molds, yeasts, and mushrooms. All of us in one way or another are influenced by fungi. Think of it for a moment - the good life without penicillin or a fine wine. Consider further the importance of fungi in the decomposition of wastes, and the potential hazards of fungi as pathogens to plants and to humans. Yes, fungi are ubiquitous and important.

Mycologists study fungi either in Nature or in the laboratory, and at different experimental levels ranging from descriptive to molecular and from basic to applied. Since there are so many fungi and so many ways to study them, mycologists often find it difficult to communicate their results even to other mycologists, much less to other scientists or to Society in general.

This Series establishes a niche for publication of works dealing with all aspects of mycology. It is not intended to set the fungi apart, but rather to emphasize the study of fungi and of fungal processes as they relate to mankind and to science in general. Such a series of books is long overdue. It is broadly conceived as to scope, and should include textbooks and manuals as well as original and scholarly research works and monographs.

The scope of the Series will be defined by, and hopefully will help define, progress in mycology.

Paul A. Lemke

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PREFACE

Genetic elements enter cells either through heredity or *via* infection. In most cases the distinction between heredity and infection is straightforward. Hereditary transfer involves only cells and occurs either at cell division or at cell fusion. Infectious transfer proceeds from the environment and involves invasion of cells by *extrinsic* genetic elements.

The distinction between heredity and infection, however, is less clear for certain genetic elements found among eukaryotes, especially in the fungi. Such *elements of infectious heredity* represent the main subject area of this book. These elements, on the basis of their physico-chemical properties and ultrastructural details, appear to be *extrinsic* to the host cell in evolutionary origin. As such, they are not an integral part of the cell's genetic makeup. However, once having entered cell lines, they persist, and remain endogenous to the cell not only in their replication but in their transmission. They have greatly reduced or even lost potential for infectivity; their lateral transmission is mediated by cell fusion; and most seem not to be detrimental to the host.

Elements of infectious heredity among eukaryotic life forms are basically of three types: *defective viruses*, *endogenous plasmids*, and *endosymbionts*.

In the fungi there is a rather large series of defective viruses that contain double-stranded RNA (*ds*RNA). Early evidence for *ds*RNA-containing viruses in fungi did not develop from genetic studies on cytoplasmic inheritance, but rather from interest in interferon induction by fungal-derived *ds*RNA. The introductory chapter of this book recounts historical developments in the study of *ds*RNA-containing mycoviruses, and considers prospects for their use in interferon in-

duction. The physico-chemical as well as the biological and ultrastructural aspects of these viruses are discussed in succeeding chapters (Chapters II-VIII). The issue of infectivity is quite important but not clearly or simply defined with regard to mycoviruses. There is only scant evidence that purified mycoviruses will infect fungal cells, and this has been acquired in the laboratory only through use of protoplasts or through artificial injection experiments. In all cases, the efficiency for infection has been poor to negligible, and the results have been difficult to reproduce. In the absence of infectivity, authors are prone to refer to *viruseslike particles* (VLP) rather than to *viruses* to designate mycoviruses. The use of VLP, however, avoids the issue of their actual nature. The term *defective viruses* is perhaps to be preferred, although all authors contributing to this volume may not agree with the use.

However, although viruses may be defective for aspects of assembly and for infectivity, they may be highly evolved in their association with the host cell. The possibility that DNA-containing viruses exist in fungi is discussed in Chapters IX and X; the latter chapter gives credence to the possibility of an obligate association of a virus with a fungus.

The term *endogenous* in reference to plasmids means that such plasmids are self-transmissible. They are not carried into cells by a phage vector but are inherited through cell-mediated plasmogamy. Among fungi, only one covalently closed circular DNA (*ccc*DNA), the 2 μm DNA plasmid of yeast, has been physico-chemically well characterized (Chapter XIII). As this book goes to press, physical evidence for *ccc*DNA in another fungus has been published (see reference 44a of Chapter XIV). An additional and recent reference (reference 28 of Chapter XIII) deserves to be mentioned in this context, as it presents irrefutable evidence for genetic transformation of yeast protoplasts by DNA using a bacterial plasmid as a vector. Thus, this study opens up the possibility to use homologous fungal plasmids as vectors for experiments in the genetic manipulation of fungi.

The precedents for *endosymbionts* in eukaryotic cells lie mainly in studies with protozoan systems, especially with reference to the killer phenomenon of paramecia. In the fungi, the evidence for *endosymbionts* is solely ultrastructural and is reviewed in this book (Chapter XII) with the prospect for expanded interest and research in the study of such elements.

One chapter of this book (Chapter XI) deals with a truly infectious agent in a fungal system, and although *ds*RNA is implicated in this system, more precise information is needed on the infectious nature of the nucleic acid and on the possibility that nondefective viruses exist in this fungus. Clearly, preliminary results with this system indicate that a more traditional approach in the study of infection will be possible with a fungal system.

I should like again in this foreword to reflect upon concepts relating to heredity and infection. Biologists tend to be quite flexible in adopting concepts that relate to heredity. Even with the rediscovery of Mendel, it was early recognized that not all genes might follow mendelian laws. Concepts for cytoplasmic inheritance were put forth, especially by Correns, and evidence for gene linkage in eukaryotic systems soon followed. With regard to concepts for infection, biologists tend to be less flexible. There is a tendency to equate infection with pathogenicity and with agents that are of necessity infectious as free elements. It is evident from the study of fungal viruses, as well as from the study of defective viruses in other life forms, that the conceptual framework for infection needs to be expanded to accommodate all elements of infection. These elements may not be pathogenic; they may be latent or even beneficial to the host cell. As such, they need not follow dictates or postulates for pathogenic infections. Rather, they can be viewed as elements of infection that, once having entered the cell, persist and usurp the methods of heredity.

Regardless of these considerations, it is clear that elements of infectious heredity must now be given serious consideration in developing fungi as genetic systems.

Paul A. Lemke

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Chapter I

FUNGAL VIRUSES, DOUBLE-STRANDED RNA AND INTERFERON INDUCTION

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I. SEARCH FOR ANTIVIRAL AGENTS

Following the discovery at Oxford in England of the striking chemotherapeutic effects of penicillin in bacterial infections in mice (34) and, subsequently, in severe clinical infections in man (1), many thousands of microorganisms were screened for antibacterial action, mainly in research laboratories of pharmaceutical companies. This huge program resulted in the discovery of many antibiotics, which together with their chemically modified derivatives, have enabled the vast majority of bacterial infections to be brought under almost complete control, however, the viral infections remained undefeated.

It was natural that the screening program which had been so successful in producing antibacterial agents should be extended to include antiviral agents. Testing for antiviral activity, however, required specialized techniques and facilities and was both costly and time-consuming. Moreover, viruses are intracellular parasites whose biosynthetic pathways are closely integrated with those of their hosts, and great difficulty was experienced in finding antiviral agents which were not also toxic to the host cells. Nevertheless, two promising antiviral agents were discovered, both from *Penicillium* species.

The first of these was discovered at the Lilly Research Laboratories in Indianapolis (USA) by Powell and coworkers (153) who found that culture filtrates of a strain of *Penicillium stoloniferum* exhibited activity against murine meningoencephalomyocarditis (MM) virus and Semliki Forest virus in mice. Further work showed that the inhibitory substance, designated 'M5-8450', was active against a wide range of virus infections in animals and in tissue cultures (151,152,95,96,36,105). Later and in

the same laboratories, a substance with antiviral and chemical properties essentially the same as M5-8450 was isolated from culture filtrates of another strain of *Penicillium stoloniferum* and was first designated 'antiviral agent 1758' (154) and then called 'statolon' (155). The new strain of *Penicillium stoloniferum* (ATCC 14586), unlike the original strain, was easily adapted to growth by submerged culture in fermentation tanks, allowing production of statolon on a commercial scale (172). Partial purification of statolon by a procedure which involved precipitation with isopropanol, treatment with sodium dodecyl sulphate (SDS) and adsorption on Hyflo Super Cel gave a product which contained, as a major component, a complex anionic polysaccharide, believed to be the active antiviral ingredient (117).

Shope (169), working at the Rockefeller Institute for Medical Research in Princeton (USA), in collaboration with the Merck Institute for Therapeutic Research in Rahway (USA), found that culture filtrates and mycelial extracts of a strain of *Penicillium funiculosum*, a strain isolated on Guam in 1945 from the isinglass cover of a photograph of his wife, Helen, contained an antiviral substance which he appropriately named 'helenine'. Helenine was found to be active against a wide range of viruses including Columbia SK encephalitis, Eastern equine encephalitis and Semliki Forest virus infections in mice (169,170) and poliovirus infections in monkeys (37). Partial purification of helenine, extracted from the mycelium of *Penicillium funiculosum* grown in 80 gallon fermenters, by a process involving acetone precipitation and ultracentrifugation indicated that the active component was a ribonucleoprotein (130,131).

II. DISCOVERY AND NATURE OF INTERFERON

Viruses and viral proteins are immunogenic and will stimulate in animals the formation of both free circulating antibodies and sensitized lymphocytes. Both mechanisms are important in the recovery from virus infections. Viruses which produce systemic disease with a plasma viremia (*i.e.*, poliovirus) are probably controlled primarily by circulating antibody, while in cases where the surfaces of infected cells contain virus-specified antigens (*i.e.*, pox and herpes viruses) control by immune lymphocytes may be more important. Previous infection with a virus may render an animal specifically immune to subsequent reinfection with

the same or closely related viruses. A similar effect may be achieved by the use of vaccination (*i.e.*, the injection of killed or attenuated live viruses)(67).

The phenomenon of viral interference in which treatment of animal cells with one virus (*the interfering virus*) protected the cells from infection with a subsequent virus (*the challenge virus*) has been known for over forty years. That such interference could occur by processes not involving specific immune reactions was clearly established when it was shown that mutual inhibition of viral replication occurred with antigenically unrelated viruses (*i.e.*, influenza and equine encephalomyocarditic viruses)(163) and in systems lacking systemic immune mechanisms, namely in the chick embryo (86) and in tissue culture (126). One widely studied system was the induction of interference to the replication of live influenza virus in chorioallantoic membrane from chick embryos by inactivated heat-treated or ultraviolet-irradiated influenza virus (204). The main advantages of this system were that (i) induction of interference by noninfective virus facilitated assay of the challenge virus and (ii) virus multiplication was limited to the endodermal layer of the membrane facilitating quantitation of the results. In these early studies it was widely assumed that complete resistance of chorioallantoic membrane to virus could be achieved only if all the cells of the membrane made direct contact with the interfering virus.

In 1957 a major breakthrough in the study of viral interference was made at the National Institute for Medical Research in London (England) by Isaacs and Lindenmann who demonstrated that when pieces of chick chorioallantoic membrane were treated with heat-inactivated influenza virus a substance was released into the medium which could be taken up by other cells (which did not have direct contact with inactive virus), rendering them resistant to challenge with live influenza virus. The inhibitory substance was given the name *interferon* (101).

It soon became clear that production of interferon is not limited to chick chorioallantoic membranes or as a response only to influenza virus. Wagner (191) found interferon in the allantoic fluid of chick embryos inoculated with live influenza virus and Isaacs and Hitchcock (100) detected interferon in the lungs of mice infected with type A influenza virus. It is now known that both RNA and DNA viruses