General Virology
3rd edition

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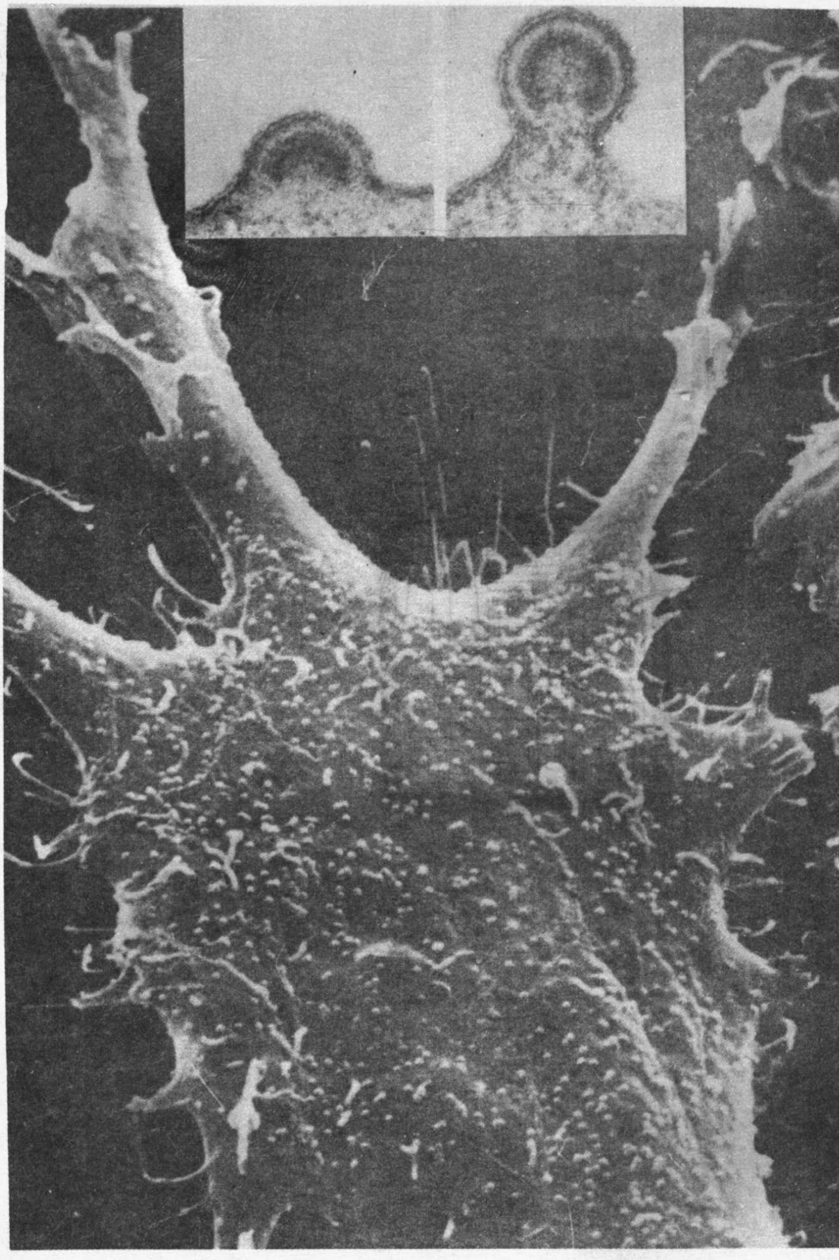

Scanning electron micrograph showing numerous murine leukemia viruses at surface of an infected cell. Inset shows section through "budding" virus particles. (Photographs courtesy of Dr. P. K. Y. Wong)

Preface

The third edition of this book appears at a time when virology as a biological science risks having its identity blurred as a result of its own successes. The growth of molecular biology, out of the twin roots of structural biochemistry and cellular genetics, both of which have fed largely on virus research, tends to obscure the limits between the basic biological disciplines.

Like all sciences that come to maturity, such as physics since Planck and Bohr or chemistry since Fischer and Pauling, biology since Watson and Crick has become unified at a finer level of specification than that provided by Darwin and Mendel. The borders between branches of biology are being overrun by the rising tide of modern cell biology. As a result, the teaching of virology as a biological science, flourishing in the 1950s and 1960s, is being dispersed among more catholic courses. Yet the very fact that virology has played so central a role in the development of molecular biology and remains a most vital field of biological and biomedical research justifies the present effort to maintain the unity of the field within a book whose previous editions have, we trust, helped catalyze the integration of virology into cell biology.

The growing sophistication of courses in general biology, genetics, and biochemistry that provide the basic preparation for biology students has made it possible to restrict the present edition of *General Virology* more precisely to its own subject by deemphasizing summaries and surveys of ancillary disciplines. This has allowed us to expand and bring up to date the strictly virological topics without increasing substantially the size of the book.

The methodological advances in the last 10 years, especially in physical biochemistry and electron microscopy, have been only peripheral to the enormous development of the field of virology in the same period. The most important advances have been, on the one hand, the flourishing of bacteriophage research as a branch of molecular genetics and, on the other hand, the role of tumor viruses and other viruses in the study of cellular biochemistry. The present picture of viral biochemistry and viral genetics promises wide advances not only in cell biology but in the study of molecular evolution.

These developments have helped to attract to virology a large influx of enthusiastic and productive researchers. The field has flourished and expanded beyond the territory that can be presented in detail within a book of this size. Fortunately the intellectual framework of the field, while expanding, has remained intact, so that it is still feasible to present an integrated and coherent overall picture. The wealth of virological literature has made it desirable for us to use as references a number of valuable and readily available monographs as well as recent reviews of specific fields. We have retained the principle of direct reference to original articles when these represent either historically significant landmarks, recent important contributions, or sources of specific methodologies and insights. As in previous editions, the choice has been dictated in large part by the inevitably fragmentary knowledge and recollections of a group of authors who are practitioners rather than chroniclers of virus research.

Collaboration between the four authors of the present edition has been made easy and fruitful by the fact that they have been associated with one another in various ways in the course of their careers. As a result they share certain views and biases as well as a common enthusiasm for virology as a frontier in the study of life. The authors are grateful to the many colleagues who provided copy for illustrations. They are especially indebted to Professor Lindsay M. Black who advised them on recent developments in the field of plant virus research and provided criticism of the parts of this volume dealing with plant viruses.

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1 Introduction: The Science of Virology

Virology as a Biological Science

Virology has become a basic biological science in its own right. Just as bacteriology emerged early this century as a biological science so did virology become, around the middle of the century, a body of knowledge and generalizations with its own perspectives and its own internal development. From its start as a branch of pathology—human and animal pathology on the one hand, plant pathology on the other—the science of virology has developed to a point where progress is dictated as much by the logic of its internal development as by the demands of applied fields.

A major stimulus to unification was the growth of knowledge about bacterial viruses or bacteriophages and the integration of bacteriophage research with bacterial genetics. A conscious effort was made by animal and plant virologists to use phage-work methods and concepts in attacking a variety of viral problems. At the same time there has emerged a new discipline, *molecular biology,* which unifies the various approaches to the structure, function, and organization of the macromolecules in which biological specificity is embodied. Virology has become an integral part of molecular biology because it deals with subcellular entities, the viruses, whose structure and organization belong in the macromolecular domain.

DEFINITIONS OF VIRUSES

The subject matter of virology, the viruses, cannot be defined by the common-sense criteria applied to animals or plants. The definition of viruses is itself somewhat arbitrary, and in fact many definitions have been proposed, an indication of the difficulty of the task.

Lwoff (1957) proposed to define viruses as "strictly intracellular and potentially pathogenic entities with an infectious phase and (1) possessing only one type of nucleic acid, (2) multiplying in the form of their genetic material, (3) unable to grow and to undergo binary fission, and (4) devoid of a Lipmann system" (that is, a system of enzymes for energy production). This definition, whose implications will become clearer in later chapters, stresses the noncellular nature of viruses, their dependence on host-cell metabolism, and the fact that at some stage of its reproductive cycle the specific material of a virus is reduced to an element of genetic material, a nucleic acid.

Another definition considered viruses to be "elements of genetic material that can determine, in the cells where they reproduce, the biosynthesis of a specific apparatus for their own transfer into other cells" (Luria, 1959). This definition stresses the independence of the viral genome, its reproduction, and its specialization for cell-to-cell transfer rather than the lack of metabolic self-sufficiency.

In the first edition of this book (Luria, 1953*a*) at a time when the nature of genetic materials was uncertain and the events of their replication and function were still obscure, it seemed desirable to employ a strictly operational definition; that is, one that would limit itself to providing certain criteria for inclusion or exclusion of given objects into the category of viruses. The definition then adopted was: "Viruses are submicroscopic entities capable of being introduced into specific living cells and of reproducing inside such cells only." A more satisfactory definition (modified from Luria and Darnell, 1967) is a composite one: *"Viruses are entities whose genomes are elements of nucleic acid that replicate inside living cells using the cellular synthetic machinery and causing the synthesis of specialized elements that can transfer the viral genome to other cells.*

This definition conveys the two essential qualities of viruses: first, the possession of specific genetic materials that utilize the biochemical machinery of the host cells; and, second, the possession of an extracellular infective phase represented by specialized objects or *virions*, which are produced under the genetic control of the virus and serve as vehicles for introducing the viral genome into other cells.

The first quality defines intracellular parasitism. This property is shared with viruses by other classes of parasites, including some bacteria, fungi, and protozoa. The definition of viruses, however, stresses the intimate nature of viral parasitism, which may be called *parasitism at the genetic level.* Parasites such as the malarial plasmodia, the leprosy bacillus, the rickettsiae, and the chlamidiae have cellular organization—a set of chromosomal genes, a ribosomal apparatus, and a mitochondrial apparatus or its equivalent, with more or less complete systems for energy release and utilization. Whether their cellular organization is that of eucaryotic cells or of procaryotic cells, these parasites are cellular organisms. Their obligatory intracellular parasitism presumably reflects requirements for exogenous supply of special nutrients or of metabolic intermediates rather than a need for a host-cell machinery through which the genome of the parasite can express itself.

The fact that virus reproduction is strictly dependent on host cells separates viruses from free-living, extracellular submicroscopic parasites, such as the mycoplasmas, which possess cellular organization and are probably stripped-down bacteria without rigid cell wall. The mutational loss of cell-wall production in common bacteria yields the so-called L forms (Smith, 1964) and provides a model of how such regressive evolution may have occurred.

The distinction between the intracellular and the extracellular forms of a virus implied in the above definition is essential. Within a host cell, disappearance of the virion as a structural entity and release of the viral genome are essential steps of the process that leads to the integration of the viral genome into the metabolic machinery of the cell and finally to replication of the viral genome. The preeminent role of viral nucleic acid in the process of virus infection has been confirmed by the fact that isolated nucleic acid molecules of certain viruses can initiate infection, as first shown for tobacco mosaic virus (Gierer and Schramm, 1956).

In this light the production of mature, complete virions has come to be considered as the culmination of a morphogenetic process directed by the viral genome. Certain genes of the virus direct the production of proteins that generate a shell or *capsid,* into which a viral nucleic acid element becomes enclosed (or "encapsidated"). Other viral proteins may complicate the form of the capsid by adding spikes or tails, as described in Chapter 3. The capsid may finally become surrounded by an *envelope* related to cell membranes but containing some virus-specific proteins. Viruses which by mutation lose the ability to produce a complete virion may persist in nature as *defective* viruses either by transmission from host to host in the form of infectious nucleic acid or by vertical spread, that is, by propagation from a virus-carrying cell to its daughter cells and even from generation to generation through the germ cells.

Even though the small size of all virions is not specifically mentioned in the definition of viruses (there is no theoretical reason why a virus could not have virions of the same order of size as the cells it parasitizes) the small size of the particles of all known viruses has played an important historical role in determining the technology of virology. Filtration has traditionally been used to separate the infectious virus particles from contaminating bacteria and to prove the nonbacterial etiology of viral diseases. Hence the traditional designation of *filtrable virus,* which replaced the earlier term of *ultravirus* and later gave way to the present usage of the word *virus.*

VIRUSES AS ORGANISMS

Are viruses living? Are they organisms? When it was found that the virions of a virus, purified and concentrated from extracts of infected cells, possess uniform size, shape, and chemical composition and can even crystallize (Stanley, 1935), the question arose of reconciling the "molecular" nature of these particles with their ability to reproduce. As usual in cases of this kind, the difficulties were semantic rather than substantial. Words such as "organisms" and "living" have unambiguous meanings only when applied to the objects for which they were originally coined: a frog is an organism, a dog that runs and barks is living. But what is it that makes a frog or a dog an organism?

According to Lwoff (1957) an organism is an "independent unit of integrated and interdependent structures and functions." A frog is such a unit; the individual cells of a frog are also units of integrated and interdependent structures and functions, but are not independent in the usual sense of