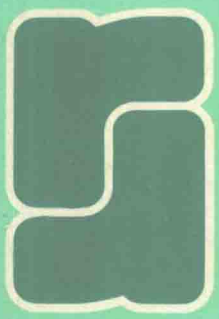

Receptors and Recognition

Series B Volume 8

Virus Receptors

Part 2
Animal Viruses

Edited by
K. Lonberg-Holm
and
L. Philipson

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Part 2 Animal Viruses

Edited by
K. Lonberg-Holm

Du Pont Central Research and Development Department, Delaware

and

L. Philipson

The Wallenberg Laboratory, University of Uppsala, Sweden

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Receptors and Recognition

About the series

Cellular recognition – the process by which cells interact with, and respond to, molecular signals in their environment – plays a crucial role in virtually all important biological functions. These encompass fertilization, infectious interactions, embryonic development, the activity of the nervous system, the regulation of growth and metabolism by hormones and the immune response to foreign antigens. Although our knowledge of these systems has grown rapidly in recent years, it is clear that a full understanding of cellular recognition phenomena will require an integrated and multidisciplinary approach.

This series aims to expedite such an understanding by bringing together accounts by leading researchers of all biochemical, cellular and evolutionary aspects of recognition systems. This series will contain volumes of two types. First, there will be volumes containing about five reviews from different areas of the general subject written at a level suitable for all biologically oriented scientists (Receptors and Recognition, series A). Secondly, there will be more specialized volumes (Receptors and Recognition, series B), each of which will be devoted to just one particularly important area.

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Contributors

J. Bramhall, Department of Bacteriology University of California, Los Angeles, U.S.A.

P. Boulanger, Laboratoire de Virologie Moleculaire de l'INSERM, Lille Cedex, France.

A.T.H. Burness, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Canada.

R.L. Crowell, Department of Microbiology and Immunology, Hahnemann Medical College, Philadelphia, Pennsylvania, U.S.A.

J. DeLarco, National Cancer Institute, National Institutes of Health, MeLoy Laboratories, Inc., Springfield, Virginia, U.S.A.

K.V. Holmes, Microbiology Department, Uniformed Services University of Health Sciences, Bethesda, Maryland, U.S.A.

N. Incardona, Microbiology Department, University of Tennessee Medical Units, Memphis, U.S.A.

B.J. Landau, Department of Microbiology and Immunology, Hahnemann Medical College, Philadelphia, Pennsylvania, U.S.A.

K. Lonberg-Holm, Du Pont Central Research and Development Department, Wilmington, Delaware, U.S.A.

L. Philipson, The Wallenberg Laboratory, University of Uppsala, Sweden.

A.S. Schied, The Rockfeller University, New York, U.S.A.

J-S. Siak, Department of Microbiology and Immunology, Hahnemann Medical College, Philadelphia, Pennsylvania, U.S.A.

C. Sigenas, Department of Microbiology, Biomedical Centre, University of Uppsala, Sweden.

R.A. Weiss, Chester Beatty Research Institute, London, U.K.

B.J. Wisnieski, Department of Bacteriology University of California, Los Angeles, U.S.A.

Contents of *Virus Receptors Part 1 Bacterial Viruses*

Preface

It is hardly necessary to define the concept of receptors to readers of this series, but it should be borne in mind that in several instances receptors are undefined entities, whose molecular details remain to be established. On the other hand the ligand, which recognizes the receptors, has been identified and characterized in most cases. The current interest in the structure and function of biological membranes gives great expectations that we may understand in the near future the details of ligand–receptor interaction. This interaction involves two defined steps; the first, usually referred to as recognition, is followed by the second step, transduction, in which the ligand–receptor interaction is translated by the cell into a biochemical action.

The present two volumes which cover prokaryotic and eukaryotic virus receptors, have been published together in order to illustrate the specificity of virus–receptor recognition which appears to be a guiding principle for both bacteria and higher cells. The identification and characterization of the receptors for phages of gram-negative bacteria has to a large extent relied on the genetic techniques available for these organisms. In a similar way the availability of genetic systems has also clarified the interrelationship between animal retrovirus receptors even if the molecular structure remains to be determined. The paucity of defined genetic systems may therefore explain part of our ignorance concerning the molecular details of virus receptors on human cells and possibly also on gram-positive bacteria. Based on evolutionary considerations virus receptors can hardly function primarily to support virus multiplication. They probably serve as important receptors for cell–cell recognition or for non-viral ligands. Some virus receptors in gram-negative bacteria have in fact already been identified as proteins involved in the uptake of nucleosides and other metabolites. In the long term a continued search for the identity of viral receptors in animal cells may therefore help to dissect specific functions of the plasma membrane.

The transduction of a receptor–ligand interaction into a biochemical signal may be complex and diversified both in prokaryotic and eukaryotic cells. This phase in the virus–receptor interaction is usually referred to as the penetration step and involves the introduction of the viral genome or the viral nucleoprotein to the site of replication. The details of these events are only now coming into focus and we can expect a rapid expansion of this field. It is premature to predict the nature of the normal transduction processes in prokaryotes and eukaryotes that are subverted by the viruses and the evidence from the prokaryotes appears to suggest that viral functions control the penetration step.

We hope that these two volumes although not comprehensive, will stimulate investigators to penetrate a neglected area of virus research, which we hope will provide as many returns as the use of bacterial and animal viruses as models for gene expression.

I want to thank my coeditors Linda Randall and Karl Lonberg-Holm who have made untiring efforts to present a coherent picture of a difficult field now experiencing a rapid development. All favourable comments about these books should however be credited to the authors and all critical comments should be directed to me.

Uppsala, February 1980 Lennart Philipson

Contents

1 Attachment of Animal Virus to Cells: an Introduction

KARL LONBERG-HOLM

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1.1 HISTORICAL PERSPECTIVE

The receptor concept has inspired progress in immunology, endocrinology, and pharmacology. This volume was optimistically conceived a few years ago to assess inchoate advances in animal virus receptor research. Despite the growing popular interest in this area, and a few notable discoveries, progress continues to be slow.

Research on the ubiquitous sialoprotein receptors for myxoviruses has continued for 40 years (Hoyle, 1968) and was stimulated by the discovery that the major erythrocyte receptor also carried MN blood group determinants (Kathan *et al.,* 1961), probably located in a separate region of the molecule (Furthmayer, 1978). However, as reviewed in Chapter 4, we do not know the precise structures recognized by different myxoviruses, and controversy remains as to the role of glycolipids as receptors (Chapter 7; Wu *et al.,* 1980). Interest in poliovirus 20 years ago led to investigation of species and organ specificity of poliovirus receptors (Holland, 1961) but presence or absence of receptors did not explain the pattern of pathogenesis in paralytic disease (Harter and Choppin, 1965; Kunin, 1964). The properties and distribution of receptors for a number of picornaviruses have now been studied, but their biological roles remain enigmatic, as reviewed in Chapter 9. Furthermore, there has not yet been adequate chemical characterization of any receptor for a non-enveloped virus (Chapter 6), and for picornaviruses a controversy about the virus capsid proteins which are engaged in receptor recognition is unresolved (Chapter 2). The components of enveloped viruses (Chapters 3 and 10) which recognize cellular receptors are well established, but with one possible exception (Fries and Helenius, 1979; Helenius *et al.,* 1978; Oldstone *et al.,* 1980) the cellular receptors for other than myxoviruses have eluded identification, as discussed in Chapter 5. Our understanding of the theoretical factors which affect the binding constants and rates of attachment of viruses to cells also remains in a primitive state, despite a major contribution almost 50 years ago (Schlessinger, 1932). Chapter 8 surveys present theoretical limitations and suggests that this may be a fruitful area for future work.

The slow rate of progress (Lonberg-Holm and Philipson, 1974, 1980; Meager and Hughes, 1979) may be partly due to lack of manpower and funding, but it is probably mostly due to technical difficulties encountered when ultrastructural analysis and protein purification methods are applied to rare components on the cell surface. It is clearly desirable that we examine the limitations of our present knowledge critically, and the expert authors of the following chapters have been encouraged to do so.

Hopefully, this book will help raise interest in virus receptor characterization, since it now appears that an adequate technology is becoming available. The introductory chapter concerns basic techniques essential for measuring attachment

of viruses to cells, and includes some comments on technical problems in detection of virus–receptor interaction during receptor isolation and characterization *in vitro*.

1.2 RECEPTOR UNITS AND RECEPTOR SITES

Discussion of structures involved in virus attachment are often confusing and the following terms are defined for increased clarity:

Virus attachment protein(s) (VAP). Virion structure(s) which can recognize a cellular receptor.
Cellular receptor unit (CRU). Cellular molecules recognizing one VAP.
Cellular receptor site (CRS). Cellular structure containing one or more CRU which can effectively bind one virion.

Although some authors reserve 'site' for a submolecular region, we feel that 'cellular receptor site' as used above is reasonably unambiguous and that the term 'determinant' can be used to clearly describe a submolecular region.

The need for these definitions results from the casual tendency to use the word 'receptor' for both viral and cellular structures. However, it is also important to emphasize that virions are constructed of repeating identical subunits (Crick and Watson, 1956) and thus can attach by multivalent bonds. This is especially likely if the cellular receptor units are mobile within a fluid membrane. Cellular receptor sites may be formed when CRUs are recruited by the virus, and thus may not exist prior to virus–cell interactions. The implications of CRS multivalency are far reaching and infer that:

(a) There are more CRUs than CRSs.
(b) *Avidity* of CRS for virus is greater than affinity of CRU for virus.
(c) Recruitment of CRU into CRS may alter the shape of a membrane lipid bilayer (Haywood, 1975; Hewitt, 1977), leading to invagination and micropinocytosis (viropexis), and possibly also to local alteration of lipid composition (Haywood, 1974).

There is little doubt that the CRS is multivalent. The average number of CRSs per cell can be calculated by studying attachment of virus particles to cell suspensions. CRU per cell can be calculated with purified and characterized preparations of VAP. For example, adenovirus fiber protein uses the same receptors as intact virions but about 10 times more fibers attach and are needed per cell for saturation of CRU. Intramembranous particles known to contain the major erythrocyte CRU for influenzavirus A are also recruited by attached virions as seen in freeze-etched replicas (Marchesi *et al.*, 1971).

Attachment of purified human enteroviruses and adenoviruses to cultured transformed cells slows significantly after 10^3–10^4 virions have been bound per cell

(reviewed by Lonberg-Holm and Philipson, 1974 and 1980). This suggests that for many viruses there may be about 10^4 CRSs and 10^5 CRUs per cell, and the latter frequency is often encountered with many specific cell membrane proteins.

Neuraminidase-sensitive receptors for certain viruses may be exceptions to this 'rule'. For example, there appear to be more than 10^5 CRSs on cultured cells for EMC virus (McClintock *et al.*, 1980), suggesting that in this case there are significantly more than 10^5 CRUs. This relatively large number of CRUs may explain the very rapid rates of attachment of cardioviruses. Alphaviruses (Birdwell and Straus, 1974; Fries and Helenius, 1979) and minute virus of mice (Schlessinger, 1932) have also 10^5 or more receptor sites on some host cells.

The number of viruses which can attach per cell is limited by availability of CRU. Typically, 10^4 enterovirus particles occupy most of the available sites on a cultured transformed host cell, but this covers less than one percent of the cell surface (Lonberg-Holm and Philipson, 1980). Viruses sharing common CRU can be identified by competition-binding experiments in which excess of one virus prevents attachment of a second virus (Lonberg-Holm and Philipson, 1980; Lonberg-Holm *et al.*, 1976; Quersin-Thiry and Nihoul, 1961), and in this way a set of virus 'receptor families' may be constructed, as reviewed in Chapters 9 and 10. Although members of a receptor family are often closely related viruses, unrelated viruses may also share receptors. Competition is generally 'all or none' in character: either two viruses share receptors or they do not influence each other's binding. However, a 'partial overlap' may be detected. For example, excess adenovirus type 2 causes a small but significant reduction in the rate of attachment of human rhinovirus type 2. Since this is detected only with adenovirus virions and not with free fibers (Lonberg-Holm *et al.*, 1976a), it may be due to steric hindrance of the HRV2 binding determinants, or alternatively may be due to the existence of two types of CRU for adenovirus virions, one for fiber and a second for capsid protein which competes for the rhinovirus CRU. There is evidence that Semliki Forest virus binds to two kinds of CRU. In this case virus under physiological conditions may attach first to histocompatability antigens (Fries and Helenius, 1979) or other membrane proteins (Oldstone *et al.*, 1980) and subsequently at slightly acid pH to membrane lipids, as discussed in Chapter 5.

There is no reason for CRU to evolve as efficient receptors for viruses, but it is of interest to consider their effectiveness from a thermodynamic point of view. Neutralization of electrostatic charges during attachment may result in a decrease in polarity and a net increase in entropy, and hence will speed the reaction. This may be one reason that sialoproteins are often selected as virus receptors. Loss of the virion's translational and rotational freedom upon attachment must decrease the net entropy and this may have a negative effect on the reaction. If CRU are elongated or flexible molecules, there will be a minimal decrease in freedom of motion during the initial step of attachment, as discussed for antibody-antigen reactions (Corothers and Metzger, 1972). Hence flexible membrane glycoproteins are more likely to be the initial CRU than are membrane glycolipids. Similarly, the rate of attachment

will be enhanced when VAP are elongated or flexible, as is the case with adenovirus fibers, or the spikes of enveloped viruses.

1.3 MEASUREMENT OF VIRUS ATTACHMENT

When virus attaches to cellular receptors it is lost from the fluid phase. Experimental measurement of this process requires critical evaluation of simplifying assumptions. For example, binding to soluble receptors or spontaneous inactivation of virus during incubation must be assessed. It must neither be assumed that receptor-bound virus has permanently lost infectivity nor that the virus remains unaltered so that it can be titrated after dissociation with a detergent or a chaotropic agent. The method used to separate free and bound virus may also alter the experimental results.

It is easier to study attachment of virus or VAP to intact cells, than to subcellular preparations. The simplest system consists of suspension-grown cells. Concentrations of HeLa cells can be as high as 5×10^7 ml^{-1}, permitting the use of relatively large amounts of virions (5×10^{11} ml^{-1} with a multiplicity of 10^4 particles per cell) and this is an advantage with isotopically labeled preparations of virions which typically contain 10^{13} particles per μCi. Of course much lower multiplicities and lower cell concentrations can be used when attachment of infectious units (pfu) are measured, since only a few thousand pfu (10^4–10^7 particles) can be used. As discussed in the next section, the rate of attachment is proportional to the cell concentration. It is thus sometimes convenient to use cells at a low concentration so that the relatively rapid attachment of certain viruses can be measured accurately. Cell monolayer cultures may also be used, and when monolayers are used, attachment of infectious viruses may be scored by counting plaques produced on the monolayer.

It is often advantageous to measure attachment at very short time intervals. This permits use of high cell concentrations and, more important, reduces artifacts resulting from processing of cell-associated virus subsequent to attachment. Manual sampling of infected suspensions of cells with an automatic pipet has been satisfactory (Lonberg-Holm and Whiteley, 1976) but it is also possible to build special equipment for sampling cell suspensions at intervals of less than one second (Lonberg-Holm, 1964). The attachment reaction can be 'quenched' by dilution with (cold) medium to allow sufficient time for separation of bound and unbound virus, because the rate of attachment depends upon the cell or receptor concentration, and often also upon the temperature (see below). Diluted and chilled samples can be sedimented at low speed and the cell pellet washed, or cells can be filtered and washed on filters to which virus does not bind (Lonberg-Holm and Whiteley, 1976). Prior treatment of filters with dilute serum often reduces nonspecific binding. Alternatively, cells may be sedimented without dilution within a few seconds in a small tabletop high-speed centrifuge (Lonberg-Holm and Whiteley, 1976) thereby avoiding dissociation of loosely bound virus during dilution. Fig. 1.1 compares kinetics of attachment of radioactive human rhinovirus type 2 to HeLa cells measured by