Interferons and Their Actions

Editor

William E. Stewart II

Interferons and Their Actions

Editor

William E. Stewart II

International Laboratories for Molecular Biology of Interferon Systems
Memorial Sloan-Kettering Institute for Cancer Research
New York, New York

A. Arthur Gottlieb, M.D.
Editor-in-Chief
CRC Immunology and Lymphoid Cell Biology Uniscience Series
Tulane University School of Medicine
New Orleans, Louisiana

Published by

CRC PRESS, Inc.
18901 Cranwood Parkway · Cleveland, Ohio 44128

PREFACE

The interferon system was for many years of interest only to a relatively small group of animal virologists. When a few of us began to imply that interferons had significances beyond blocking virus replication, we spoke cautiously. Dr. Ion Gressor (Villejuif, France), Dr. George Svet-Moldavsky (Moscow), and Dr. Kurt Paucker (Philadelphia) deserve much credit for broadening the horizons of interferon research. The various actions of interferons described in this book have added much excitement to interferon research and have motivated cell biologists, molecular biologists, oncologists, and immunologists as well as virologists to search for literature on this topic; I hope this volume will provide some of the sought-after information and that the reader will share in the excitement of interferon research.

I was first introduced to interferon research as an undergraduate at Texas Tech University by Professor Lyle Kuhnley, whom I have admired for the special pleasure he takes in helping his students develop interests in basic research problems. I was then encouraged further into interferon specialization by my dear friend, Professor S. Edward Sulkin (deceased) at Southwestern Medical School in Dallas, whose wisdom, gentle nature, and patience were an example and joy for all who had the fortune to know him. I was extremely fortunate to study interferon under Dr. Royce Z. Lockart, Jr., both as a graduate student at the University of Texas, Austin, and for 2 years as his post-doc at the DuPont Experimental Station, Wilmington, Delaware; his uniquely blunt critical evaluation of data kept me out of scientific quagmires more than a few times. Professor Pete DeSomer was kind enough to let me work on my interferon projects in his labs at the Rega Institute (Louvain, Belgium) for 4 years, and when I wore out my welcome there, my friend and colleague Professor Jan Desmyter (Louvain University) graciously allowed me to contaminate his hepatitis labs with interferonology. I was then privileged to spend a year in Dr. Ion Gressor's labs (Villejuif) prior to establishing my labs at the Sloan-Kettering Institute. I have been exceptionally fortunate in sharing the enthusiasm and talents of my research technicians, Randy Rosenthal (Dallas), Elizabeth Viscusi (Wilmington), Frieda DeMaeyer and Willy Zeegers (Louvain), and Sylvia LeGoff (Villejuif).

William E. Stewart II
Sloan-Kettering Institute
New York, New York

THE EDITOR

William E. Stewart II, Ph.D., is Principal Investigator and Co-director of the International Laboratories for Molecular Biology of Interferon Systems (ILMBIS), Memorial Sloan-Kettering Institute for Cancer Research, New York.

Dr. Stewart received his B.S. degree in 1964 from Texas Technological College, Lubbock, and his M.S. and Ph.D. degrees in 1966 and 1969, respectively, from the University of Texas Southwestern Medical School, Dallas. He was a postdoctoral fellow in the Department of Virology, Central Research Division, DuPont Experimental Station, Wilmington, Delaware, before becoming Visiting Scientist and then Visiting Professor of Virology at the Rega Institute, University of Louvain, Belgium in 1971 and 1974, respectively. He was a Senior Fellow of the European Molecular Biology Organization while working at the Institute for Scientific Research on Cancer, Villejuif, France.

Dr. Stewart's work has been supported by the Damon Runyon-Walter Winchell Fund for Cancer Research, the Jane Coffin-Child Fund for Medical Research, the European Molecular Biology Organization, and the National Institutes of Health.

Dr. Stewart is a member of the American Association for the Advancement of Science, the American Society for Microbiology, the Society for General Microbiology, the European Molecular Biology Organization, and the Federation of European Biochemical Societies. Dr. Stewart's present research concerns biochemical virology, pathogenesis of viral diseases and cancer, and eukaryotic cell regulatory mechanisms. He is currently the recipient of a 3-year grant from the National Cancer Institute for the characterization of interferons and their actions. He has made many presentations at national and international meetings and has published many research papers.

CONTRIBUTORS

Esther H. Chang, Ph.D.
Visiting Scientist
Laboratory of Experimental Pathology
National Institute for Arthritis, Metabolism, and Digestive Diseases
National Institutes of Health
Bethesda, Maryland

Bud Colby, Ph.D.
Core Member
International Laboratories for Molecular Biology of Interferon Systems
Memorial Sloan-Kettering Institute for Cancer Research
New York, New York

Lois B. Epstein, M.D.
Chief, Laboratory of Experimental Pathology
National Institute for Arthritis, Metabolism, and Digestive Diseases
National Institutes of Health
Bethesda, Maryland

Robert M. Friedman, M.D.
Chief, Laboratory of Experimental Pathology
National Institute for Arthritis, Metabolism, and Digestive Diseases
National Institutes of Health
Bethesda, Maryland

Edward A. Havell, Ph.D.
Research Associate Professor
Department of Microbiology
New York University School of Medicine
New York, New York

David W. Hutchinson, Ph.D.
Senior Lecturer
Department of Molecular Sciences
University of Warwick
Coventry, England

Mathilde Krim, Ph.D.
Associate Member
Memorial Sloan-Kettering Institute for Cancer Research
New York, New York

S. H. S. Lee, Ph.D.
Associate Professor
Department of Microbiology
Dalhousie University
Halifax, Nova Scotia

Paula M. Pitha, Ph.D.
Associate Professor of Oncology
The Johns Hopkins Oncology Center
Baltimore, Maryland

K. R. Rozee
Head, Department of Microbiology
Dalhousie University
Halifax, Nova Scotia

F. Kingsley Sanders
Member
Memorial Sloan-Kettering Institute for Cancer Research
New York, New York

William E. Stewart II, Ph.D.
Co-director
International Laboratories for Molecular Biology of Interferon Systems
Memorial Sloan-Kettering Institute for Cancer Research
New York, New York

Y. H. Tan, Ph.D.
Associate Professor
School of Medicine
University of Calgary
Alta, Canada

TABLE OF CONTENTS

Chapter 1

INTERFERON SYSTEMS – AN OVERVIEW

Bud Colby

TABLE OF CONTENTS

I. INTRODUCTION

The purpose of this chapter is to define the interferon system, to discuss the molecular and genetic components involved in the induction and action of interferons, to describe the various effects of interferons at the cellular and organismal level, and to identify the application of interferon and interferon inducers with respect to certain viral diseases and malignancies. In the section summarizing the present knowledge of the molecular and genetic components of the interferon system, I shall distinguish between those which have been demonstrated experimentally and those which have only been postulated to date. Most of the interferon system is covered in the other chapters, and the technical details and references are provided therein. Thus, this first chapter is intended to provide an overview to orient newcomers in the interferon field as well as a brief review for those already familiar with the subject.

Interferons are a family of inducible proteins produced by eukaryotic cells in response to viral infection and a number of other stimuli. Their synthesis is regulated at the level of transcription and possibly at translation, and the newly synthe-

sized interferons are processed and released from the induced cells. Cells treated with interferon become resistant to infection by a wide spectrum of viruses. This resistance is the basis of current methods for the quantitative assay of interferons. It must be stressed that although they were first identified through the resistance to viral multiplication which they can induce in cells, interferons are not in themselves antiviral substances. They have no direct effect on viruses, their antiviral activity being entirely mediated by cells in which they induce the antiviral state. In addition, a number of nonantiviral activities of interferons have been described.

All species of fish, amphibians, reptiles, birds, and mammals tested have been found to produce and respond to interferons. Thus, interferons are a family of proteins for which genetic information has been conserved throughout the independent evolution of a broad spectrum of vertebrate species; this attests to the fundamental role of interferons in essential biological processes.

An interferon system can be defined as the following complex chain of molecular events:

1. The specific recognition of an interferon-inducing molecule
2. The activation of transcription of the previously unexpressed genetic information for interferon messenger RNA
3. The regulated processing and translation of interferon messenger RNA
4. The modification and subsequent secretion of the interferon protein
5. Interferon's interaction with and recognition by a susceptible cell
6. The activation of transcription of another previously unexpressed genetic information
7. The translation of this transcript
8. The alteration of the cell's metabolism, expressed as one or more of the identifiable interferon actions

Thus, the interferon system can be envisioned as events taking place in two different cells (Figure 1). In each cell, sequentially, a modification of the patterns of genetic expression occurs as a result of a defined cell surface event which appears to activate the transcription and translation of particular genetic sequences. There are also regulatory genes whose products exert controlling functions to which the temporally limited activations of this system, i.e., both that of induction of interferons and by interferons, can be attributed.

TABLE 1

Molecular and Genetic Components of the Interferon System

A. Induction of Interferon Synthesis
   1. Interferon-inducer molecule
   2. Interferon-inducer molecule recognition
   3. Interferon structural gene
   4. Interferon regulatory gene(s)
   5. Interferon messenger RNA
   6. Regulatory molecule(s)
   7. Interferon

B. Cellular Response to Interferon Treatment
   1. Interferon
   2. Interferon recognition
   3. Structural gene(s) for interferon response
   4. Messenger RNA(s) for response
   5. Regulatory gene(s)
   6. Regulatory molecule(s)
   7. Antiviral protein
   8. Nonantiviral response protein(s)

II. COMPONENTS OF THE INTERFERON SYSTEM

The biochemical and genetic components of the interferon system which have been either detected or postulated to date are listed in Table 1. For conceptual convenience, the system has been divided into the categories of interferon induction and interferon action, a practice often employed in the research laboratory and in interferon symposia. One should understand that this division is more apparent than real, as most normal cells are capable of both synthesizing and responding to interferons. I shall now abstract the experimental evidence for each of these components.

A. Interferon Inducers

A wide variety of viruses, microorganisms, bacterial and fungal products, nucleic acids of both synthetic and natural origin, polymers, and low molecular weight substances has been shown to elicit the production of interferons in cultured cells and/or whole organisms. Ho and Armstrong[1] recently presented the useful classification scheme in which Class A inducers are defined as those capable of stimulating at least 10^3 units/ml of interferon in either cell cultures or the blood-

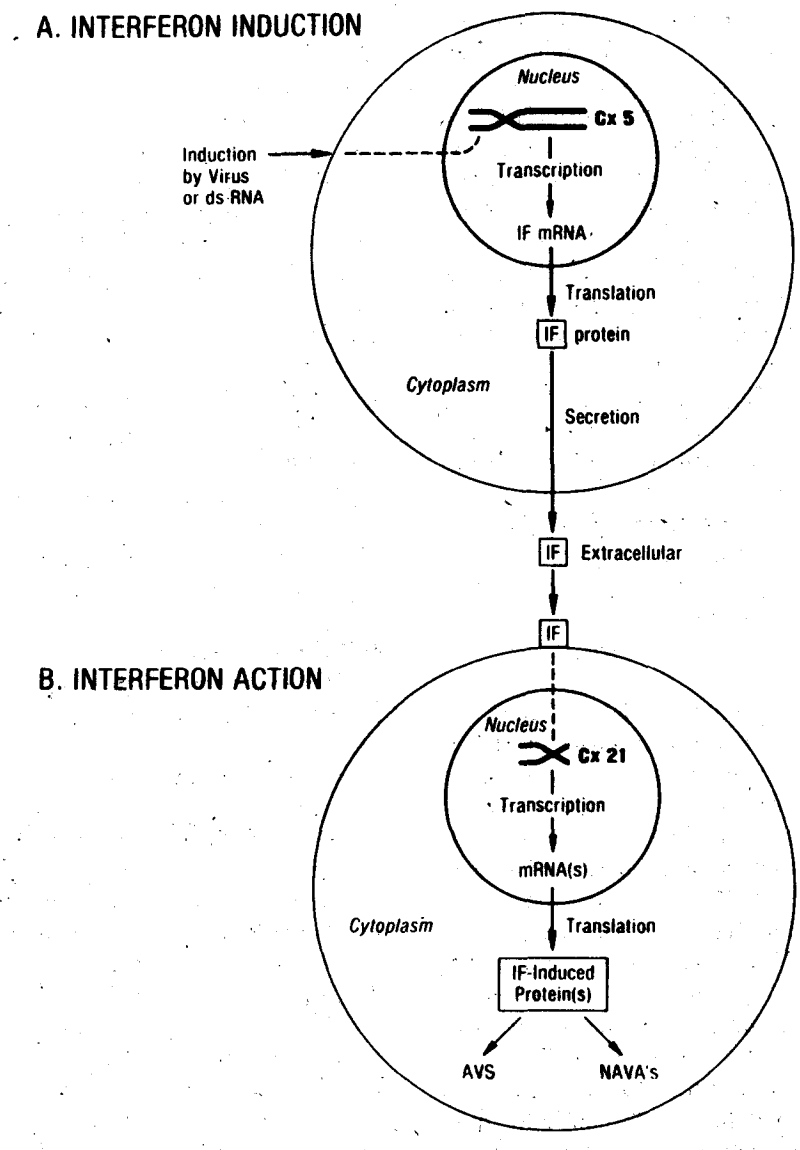

FIGURE 1. The Interferon system. The example chosen here is for the production and action of human fibroblast interferon, as the chromosomal assignment for other interferon systems has not been resolved.

stream of animals when administered in microgram quantities; Class B inducers, on the other hand, are often inactive in cultured cells and are inferior both in terms of the amount of interferon induced (usually much less than 10^3 units/ml) and the amount of inducer required, which may reach toxic (milligram) quantities. Ho and Armstrong classify separately those inducers of interferon in lymphocytes and/or macrophages which result from stimuli such as specific antigens and/or mitogens.

All Class A inducers either themselves contain double-stranded RNA or, in the case of viruses, apparently direct the synthesis of double-stranded RNA at some stage during their intracellular replication. If the designation of "inducer" is used in its genetic sense, i.e., is applied only to those molecules which initiate specific events resulting in the *de novo* synthesis of a particular protein, double-stranded RNA appears the most likely candidate for the role of the interferon-inducer molecule. The experimental evidence on which this statement rests is presented in detail by Pitha and Hutchinson in Chapter 2.

B. Recognition of the Interferon-inducer Molecule

An inducer's specific capacity to induce interferon in cultured cells appears to require the physical property of helicity, and particularly the chemical property of possessing 2′-hydroxyl groups on the sugar moieties, of both natural and synthetic polynucleotides. Accordingly, a proteinaceous receptor molecule capable of specifically recognizing these characteristics has been postulated. To date, such a receptor has not been isolated, since the purification of any molecule depends absolutely on the availability of a specific assay system for it. The assay system, in this case, would have to include the interferon structural gene, specific transcriptase activity, the molecule(s) involved in the regulation of this transcription, and a detectable transcription product. Such a system is beyond the current level of eukaryotic molecular-biological technology; only the last component is available (see the section below on interferon messenger RNA).

C. Interferon Structural and Regulatory Genes

In recent years, chromosomal assignment has become possible both for the genetic locus whose transcription is initiated by the interaction of interferon inducers with inducible human cells and for the locus involved in the regulation of human interferon synthesis. The structural gene for human fibroblast interferon has been assigned to the long arm of chromosome 5 and genetic regulatory elements to the short arm of chromosome 5. The experimental approaches involved are discussed in detail by Tan in Chapter 5.

Somatic cell genetic studies which employed fusion of murine and human fibroblasts to produce hybrids from which human chromosomes were preferentially, although randomly, lost initially showed that the ability of such hybrid cells to produce human interferon correlated with the retention of human chromosomes 2 and 5. Recent experiments with human-hamster hybrids indicate that only human chromosome 5 is required for the production of human interferon. Gene dosage experiments measuring interferon yields obtained from various human aneuploid cells, mono-, di-, or polysomic for chromosome 5 or for one of its arms, have suggested that the interferon structural and regulatory loci are on the long and short arms, respectively, of chromosome 5. Finally, a study of interferon yields by human cell lines with various translocations or deletions involving chromosome 5 has permitted the mapping of these genetic elements within narrow chromosome regions.

Using inbred mouse strains and Mendelian analysis, an attempt was made to assign the loci for interferon production induced by viruses to known linkage groups. In this way, it could be shown that the genetic locus involved in interferon production in response to induction by Newcastle disease virus segregated independently from the locus involved in the response to induction by mouse mammary tumor virus. These results suggest the existence of more than one structural gene for interferon in the mouse species. It has not yet been ascertained whether a similar situation exists in man, since the genetic assignments reported above for the human species were derived from experiments carried out with one cell type only, human fibroblast cell strains and aneuploid cells derived from them.

D. Interferon Messenger RNA

The existence of messenger RNAs for interferons has been demonstrated in several ways, all of which take advantage of the so-called "species specificity" of interferons. In 1972, DeMaeyer-Guignard et al.[2] reported the following experiment: cultured mouse cells were induced to synthesize interferon by treatment with synthetic double-stranded polyribonucleotide, $(rI)_n \cdot (rC)_n$; messenger RNA preparations from these cells were applied to cultures of chick embryo fibroblasts; the latter were then shown to produce interferon activity capable of inducing the antiviral state in mouse, but not in chick cells. Thus, the messenger RNA for mouse interferon had been taken up and correctly translated by the chick embryo cells, and the mouse interferon produced was subsequently released from the chick cells into the culture media. This original observation has been confirmed by other investigators. The translation of human mRNA preparations by heterologous cells has since been used to demonstrate the existence of human fibroblast interferon mRNA. Translation of human interferon mRNA preparations has also been accomplished by microinjected *Xenopus* oocytes and in vitro protein-synthesizing systems using Ehrlich ascites cell and wheat germ extracts.

Techniques enabling one to assay for the presence of interferon messenger RNA are currently being used by a number of research laboratories to investigate various aspects of the regulation of interferon synthesis. These assay systems

are also being used to purify and characterize interferon messenger RNAs. It has recently been shown that human fibroblast interferon messenger RNA contains polyadenylic acid sequences at the 5'-terminus of the molecule. Thus, it may eventually be possible to achieve an obligo-dT-primed, reverse transcriptase-catalyzed production of interferon-complementary DNA; the cloning of such cDNA using recombinant DNA technology; and the large-scale production of human interferon for both basic and clinical research. The present status of interferon mRNA research is reviewed by Pitha and Hutchinson in Chapter 2.

E. Regulatory Molecules Involved in Interferon Synthesis

The synthesis of interferons represents the expression of genetic information that is normally silent. Since interferon mRNA is not detectable in uninduced cells, it appears that a regulatory mechanism is acting at the level of transcription. When cells are treated with an appropriate interferon inducer, interferon mRNA becomes detectable, and interferon synthesis begins, reaches a maximum within times ranging from 4 to 36 hr (depending on the cell-inducer system), and then declines.

In many cell systems and organisms, a period follows during which cells remain refractory to reinduction. Following the induction and decline of interferon synthesis, normally several days must pass before cells can respond once more to treatment with interferon inducers (double-stranded RNA or virus), suggesting an interferon-mediated regulation of interferon synthesis.

If actinomycin D, an antimetabolite which inhibits DNA-dependent RNA synthesis, is added to cultures prior to, coincident with, or immediately following treatment with an interferon inducer, interferon synthesis does not occur. However, the addition of actinomycin D during the development of interferon synthesis results in elevated and prolonged interferon production. This phenomenon, known as "super induction," has been interpreted as possible evidence for the synthesis of an interferon-induced regulatory protein that manifests itself at the level of interferon mRNA translation. Superinduction would result from the inhibition of the transcription of the postulated regulatory gene. In Chapter 3, Havell describes the use of antimetabolites in superinduction and the mechanisms for interferon synthesis and regulation which these effects suggest.

F. Interferons

The final components in the inductive portion of the interferon system are interferons themselves. The interferon molecules are small, single-chain polypeptides which may be glycosylated and which are endowed with a number of extraordinary properties. Some interferons are capable of withstanding what most protein biochemists would consider cruel and unusual punishment, such as prolonged treatment at pH2 in the cold, heating at 56 to 60°C for 30 to 60 min, and boiling in the presence of SDS, 5 M urea, and mercaptoethanol. Consequently, many investigators envisioned the rapid and effortless purification of interferons to homogeneity and their subsequent characterization.

However, interferons also possess two other properties, one quite remarkable and one common to many proteins, which have combined to frustrate attempts at complete purification. The remarkable property is the very high specific activity. It has been estimated at about 10^9 units/mg protein; thus, even 10^6 units of interferon activity corresponds to very little interferon protein. A unit of interferon is often defined as the reciprocal of the dilution of an interferon preparation which can reduce the plaque-forming capacity of a vesicular stomatitis virus preparation by 50% in a given cell type. The property of interferons common to many proteins is their aversion to existence in a very dilute condition. The net result is that after significant purification of interferon preparations investigators have found themselves with a solution containing almost undetectable amounts of protein which rapidly becomes denatured. It is now clear that in order to effect the complete purification and characterization of interferons, one must have access to much larger amounts of starting material than have usually been available so that the final product can contain sufficient material both to resist inactivation and to be assayable by biochemical or serological methods.

No interferon has been completely purified; investigators are still working with relatively crude preparations, even when their specific antiviral activity is very high, e.g., 10^7 to 10^8 units/mg protein. Nevertheless, a certain degree of characterization has been achieved. It is known

not only that interferons derived from different animal species vary in molecular weight, but that the interferon produced by one kind of cell may differ from that produced by another derived not only from the same species, but from the same individual organism. Furthermore, present evidence indicates that interferon preparations from a single source can contain different biologically active molecular forms that can be distinguished physically, chemically, biologically, and antigenically. Preparations of human leukocyte interferon, for example, contain populations of interferon molecules differing in each of these respects from those produced by human fibroblasts.

Interferon proteins also vary in the extent to which they are glycosylated, but it is not clear whether glycosylation is entirely responsible for molecular heterogeneities, nor whether it is related to biological activity or to cell specificity. Molecular heterogeneities, either among interferon preparations produced by different cell types of the same species, or within a single preparation produced by one cell type, may originate in each case through two different mechanisms. Posttranslational modifications may occur, such as proteolytic cleavage and/or variation in glycosylation, each of which may differ in kind and extent. Another possibility is the existence in the same species of a number of different structural genes for interferons, whose activation is selective and reflects the differentiated state of each cell type. If this is the case, the interesting possibility exists that there may be tissue-specific *action* of interferons. For instance, human leukocyte and human fibroblast interferon may exert their activities on cells of different human tissues. The availability of methods for the translation of interferon mRNAs in heterologous systems now makes it possible to clarify the question of interferon's heterogeneity; such experiments are currently in progress. Present knowledge regarding the characterization of interferon preparations is reviewed in detail in Chapter 4.

The word "interferon" appears twice in Table 1; it is listed as the seventh component under the heading "Induction of Interferon Synthesis" and as the first component under the heading "Cellular Response to Interferon Treatment." This has been done to emphasize that interferon, the product of the first induction, initiates the second inductive event in the interferon system, e.g., that interferon is an induced inducer.

G. Interferon Recognition

In a general sense, interferons exhibit what has usually been called "species specificity." That is, human interferon is only slightly active on mouse cells and mouse interferon is apparently not active on chick cells. However, there are many examples of high degrees of cross-species activity (see Chapter 4). It is, therefore, more accurate to say that interferons can be characterized by their host range, i.e., the species in which they act and their efficiency of crossing species barriers.

The available experimental evidence is most easily interpreted if it is assumed that interferons interact with specific receptor molecules located at the cell surface. Furthermore, the nature of the specificity of interferon action suggests that the receptor molecule is most probably a protein. As is the case for the receptor for interferon inducers, the receptor for interferon has not been characterized and awaits the development of a suitable assay system.

H. Structural Gene(s) for Interferon Responses

The establishment of the interferon-induced antiviral state does not occur in the presence of antimetabolites which inhibit either cellular transcription for new RNA or translation of new protein. Recently, the cell's nucleus has been shown to be essential for the expression of interferon's antiviral activity. Thus, not only does induction *of* interferons require the expression of genetic information, but so does induction *by* interferons.

I. Messenger RNA(s) for Interferon Responses

Messenger RNA(s) involved uniquely in the cellular responses to interferon treatment is postulated to exist on the basis of accepted concepts of the mechanisms by which macromolecules are synthesized in cells. That is, interferon treatment does not induce the antiviral state in cells treated with antimetabolites which inhibit the synthesis of RNA or protein in enucleated cells. The experimental demonstration of such mRNA(s) will require appropriate assay systems: either a purified gene with which to perform cDNA/RNA hybridization experiments, or a product of the translation of such mRNAs which can be assayed. At present, neither assay system is available.

J. Regulatory Genes and Gene Products

The treatment of susceptible cells with inter-

ferons alters the cells so that, for example, virus replication may be reduced in them by a factor of 10,000. Since this effect takes place following *de novo* expression of genetic information, one must postulate the existence of regulatory genes which are normally responsible for the suppression of the required genetic information in non-interferon-treated cells. The genetic information regulating interferon action in human cells has been tentatively assigned to chromosome 16. However, no assay exists as yet for the product of this regulatory gene(s).

The regulatory mechanisms which control the establishment, maintenance, and decay of the interferon-induced antiviral state (AVS) appear to be quite different from those which control the synthesis of interferon, since there is no refractory state to induction(s) *by* interferons comparable to that for induction *of* interferons. The antiviral effect of interferons is concentration dependent; both the final level achieved and the rate of establishment of the AVS are dependent upon the concentration of interferon present. Furthermore, cells in the process of establishing the AVS respond to increases in interferon concentration by increasing proportionally both the rate of establishment and the final level of the AVS.

The AVS induced by interferon is maintained in the presence of interferon. However, when interferon is removed, a rapid exponential decay of resistance to virus multiplication follows; the rate of this decay is independent of the level of AVS previously established. At any time during either the maintenance or the decay of the AVS, cells are capable of responding to interferon treatment by elevating their antiviral state.

K. Interferon-induced Antiviral Protein

The antiviral protein is yet another molecular component of the interferon system whose existence seems certain but whose demonstration awaits an assay system which will allow experimental manipulation. Such an assay must be based on an understanding, at the molecular level, of the mechanisms by which virus replication is suppressed during the interferon-induced antiviral state.

Generally speaking, most of the current evidence is consistent with the interpretation that interferons affect virus replication at the level of synthesis of viral macromolecular components, that is, at transcription and/or translation of viral genes. An exception seems to be the murine leukemia viruses. Those viral proteins which have been studied (P30; reverse transcriptase) are synthesized in infected, interferon-treated cells, but virions do not appear to bud from the cell surface. The particular antiviral effect in this case seems to be limited to virus assembly or release. One or a few viral proteins, necessary for these processes but as yet unidentified, may be uniquely inhibited, or the effect may result from an interferon-induced cell surface modification. Whatever the case may be, mouse interferon is a potent inhibitor of the multiplication and spread of mouse murine leukemia viruses both in vitro and in vivo.

The work of Oxman and colleagues, described in Chapter 8, indicates an inhibition of early transcription in the lytic infective cycle of SV40 virus in interferon-treated cells. More recently, Revel,[5] using the same system with a different experimental design, has obtained evidence for the inhibition of viral translation in the absence of an inhibition of transcription.

Most other cell-virus systems studied exhibit an interferon-induced inhibition of the translation of viral messenger RNA both in infected cells and in cell-free extracts. The exact molecular mechanism by which translation is inhibited is unknown at this time; however, a variety of suggestions have been made by workers in the field. These include the appearance of new small RNA molecules, the appearance of a new ribosome-bound protein that inhibits elongation, the disappearance of certain minor species of transfer RNA required for translation, and the inhibition of initiation of translation of viral messenger RNAs. Quite recently, the laboratories of Kerr, Lengyel, and Revel independently reported that extracts of interferon-treated cells contain an ATP-dependent, double-stranded RNA-activated protein kinase.[3-5] These reports are reminiscent of the earlier work of Farrell et al.,[6] who first demonstrated that the inhibition of globin synthesis in reticulocyte extracts by double-stranded RNA is the result of the activity of an ATP, double-stranded RNA-dependent protein kinase that sequentially phosphorylates a 67,000-dalton protein and a 35,000-dalton protein. The latter has been identified as the initiation factor E2. Furthermore, Brown et al.[7] have reported finding an ATP, double-stranded RNA-dependent nuclease in extracts of interferon-treated cells. If further experimentation confirms these activities central to the mechanism of the interferon-induced anti-

viral state, we may have the two major portions of the interferon system: the induction of interferon synthesis and the establishment of the interferon-induced antiviral state, united by the helix of double-stranded RNA.

L. Nonantiviral Interferon Activities

It is now recognized that interferon treatment initiates a number of cellular alterations other than the antiviral state. Nonantiviral alterations also appear in some cases to require the expression of previously silent genetic information. One of the most important unanswered questions in the biology of interferon systems is whether the "nonantiviral activities" of interferons are mediated by the same molecule(s) and the same mechanism(s) as those which operate to restrict virus replication. Experimental results are discussed in Chapters 6 and 7. This section will briefly review these various other activities of interferons.

1. Effects on Interferon Synthesis (Priming and Blocking)

Treatment of cells with interferon preparations alters their capacity to synthesize interferon. Cells pretreated with small concentrations of interferon often produce increased amounts of interferon when subsequently induced by double-stranded RNA or viruses. This phenomenon has been referred to as "priming." By contrast, cells pretreated with large concentrations of interferon preparations often fail to synthesize interferon when subsequently treated with interferon inducers, a phenomenon known as "blocking." These two effects involve mechanisms which differ from each other in a fundamentally important way: blocking requires the expression of genetic information, while priming appears to result from a directly induced alteration of the cell surface, which does not require the synthesis of novel gene products.

2. Enhanced Toxicity of Double-stranded RNA

At concentrations far in excess of those required for the induction of interferon synthesis, double-stranded RNA is cytotoxic to certain strains of mammalian cells growing in culture. Pretreatment of these cells with interferon increases their sensitivity to the toxicity of dsRNA. That is, smaller concentrations of dsRNA result in cell death. Similarly, several laboratories have reported that interferon-treated cells show an enhanced sensitivity to the toxicity of vaccinia virus infection. It is known that the primary transcription by the virion-bound transcriptase of vaccinia virus is stimulated in interferon-treated cells and that both single- and double-stranded RNA result from the activity of this enzyme. The mechanism by which these effects occur is unknown. There are several interesting possibilities, including an interferon-mediated alteration of the cell surface membrane and an interferon-induced dsRNA-dependent inhibitor of protein synthesis and/or nuclease activity.

3. Effects on Cell Surfaces

Treatment of murine L cells with interferon has been reported to increase their electrophoretic mobility. Mouse interferon enhances the expression of histocompatibility antigens in both mouse lymphoid (thymocytes and splenic lymphocytes) and L1210 cells.

4. Effects on Cell Growth

For certain lines of human and mouse cells in culture, treatment with homologous interferon can result in (a) a decrease in the rate of cell division, both in cells grown as monolayers and in suspension, (b) a lowering of the cell saturation density of transformed cells, (c) an inhibition or complete loss of the ability of mouse tumor cells or human lymphoblastoid cells to form colonies in a semisolid matrix, (d) an inhibition of the blastogenic response of both mouse and human lymphocytes to allogenic cells and to mitogens, and (e) an inhibition of liver regeneration in vivo.

5. Effects on Expression of Cellular Functions

Certain cell modifications caused by interferon preparations appear to be of an inductive nature. However, they may involve only an augmentation of a previously low level of transcription and translation, rather than *de novo* induction, as is the case for the antiviral state. Hemoglobin synthesis is augmented in interferon-treated Friend cells exposed to DMSO. Interferon treatment also enhances the induction of lipopolysaccharide by the synthetic steroid dexamethasone and it increases the production of the enzyme aryl-hydrocarbon hydroxylase. The increased expression of H2 histocompatibility antigens on the surface of mouse lymphoid and L1210 cells, which occurs in vitro and in vivo, could involve augmented synthesis of antigens rather than uncovering of antigenic sites.

Mouse and human interferons have also been reported to affect the specialized cell functions of both T and B lymphoid cells. Their interactions with these cells have been reported to result in modulation of a number of immune responses, both in vitro and in vivo. Responses inhibited include:

1. Blastogenesis induced by allogeneic cells
2. Primary antibody response to sheep red blood cells
3. Allograft rejection
4. Graft vs. host reaction
5. Delayed hypersensitivity reaction
6. Tumor cell rejection (enhancement of tumor growth)

Responses enhanced include:

1. Primary antibody response to sheep red blood cells
2. Specific cytotoxicity of lymphocytes
3. Phagocytosis by macrophages
4. Specific tumor cell rejection

Chapter 6 of this volume will review in detail the conditions under which the above effects have been observed. The unique immunomodulatory activity of interferons seems to depend on the timing of interferon treatment relative to the initiation of the immune response as well as to the dose of interferon used. Interaction between interferons and either T or B cells appears inhibitory when it occurs early in the course of an immune response – possibly through the inhibition of the lymphoproliferative reaction to the antigenic stimulus, thus impeding the amplification of the immune response – and enhancing when it occurs late during the immune response, through increased expression of the specialized functions of immune cells.

Furthermore, as will also be described in Chapter 6, lymphoid cells responding to mitogens or to specific antigenic stimulation are induced to produce lymphokines, among which there is a viral inhibitor which, according to most criteria, qualifies as an interferon. However, this inhibitor has been found to have properties of sensitivity to low pH quite different from those of interferons produced by the same or other cells when they are stimulated by viruses or the synthetic polyribonucleotide poly rI·poly rC. This interferon has been named Type II interferon to distinguish it from the Type I interferon that is induced by double-stranded RNAs and viruses. In man, Type II interferon exerts its inducing effect on cells through a locus very close, or identical, to the gene (located on chromosome 21) which is activated by interferon Type I.

III. IN VIVO PROPHYLACTIC AND THERAPEUTIC STUDIES WITH INTERFERONS

A. Antiviral and Antitumor Trials in Animals

Interferons and interferon inducers have been used in an enormous number of antiviral and antitumor studies in animals. Several general concepts have emerged. In most viral infections the effect of interferon treatment is far more impressive when it is used as a prophylactic rather than a therapeutic agent; therefore, it probably has a more significant role in early stages of viral diseases. Thus, interferon treatment prior to or concomitant with virus infection usually results in a less severe disease, lower titers of virus in the bloodstream or infected organs, and higher survival rates. Details of these studies are presented in Chapter 9.

Recently, Gresser and co-workers[8] using quite a different experimental design, demonstrated convincingly that interferon is an important element in the response of the mouse to several viruses exhibiting different pathogeneses. Rather than treating mice with preparations of mouse interferon, these workers used a potent preparation of sheep antibody active against mouse interferon. The anti-mouse-interferon globulin apparently neutralized the endogenous interferon induced by these viruses and resulted in a highly accelerated development of the infectious processes. With encephalomyocarditis virus, for example, the animals treated with anti-mouse-interferon globulin had high-titer virus in visceral organs within 24 to 36 hr as opposed to low titer, and died within 48 hr rather than after 4 to 5 days. Similarly, following herpes simplex virus (HSV) infection, the latent period was shortened and the overall LD50 was decreased several hundredfold when compared to that of the control mice. When HSV was given subcutaneously, all anti-mouse-interferon globulin-treated animals died; only 5% of the controls died. Similarly, anti-interferon

globulin treatment resulted in an accelerated onset of disease and increased death in adult mice inoculated with vesicular stomatitis virus or Newcastle disease virus. In contrast, this treatment had no effect on influenza virus infection in mice. Furthermore, anti-interferon globulin treatment resulted in an earlier appearance of murine sarcoma virus-induced tumors, a greater number of mice bearing tumors and an increase in tumor size.

The growth of many tumors can be either inhibited or prevented by interferon treatment. These include reticulum cell sarcoma, Lewis lung tumor, spontaneous and carcinogen-induced leukemias, mouse mammary tumor and both DNA and RNA tumor virus-transformed cells.

B. Antiviral and Antitumor Trials in Man

Human interferon is apparently modestly effective in decreasing the severity of rhinovirus infection in man and is currently being used in various other clinical studies with encouraging preliminary success. For instance, the effect of human leukocyte interferon on hepatitis B virus infection in patients with chronic active hepatitis has been reported recently.[9] Interferon treatment resulted in a rapid and reproducible decrease in the levels of circulating Dane-particle-associated DNA. These effects, while transient for interferon treatment periods of 10 days, appeared more permanent with extended periods of therapy. Thus, interferon may be useful in limiting carrier infectivity or eradicating chronic hepatitis, which is currently the fate of about 10% of hospitalized hepatitis victims in the United States and is a major problem for blood-collecting centers worldwide.

The possibility of using interferon in the treatment of human cancer is now undergoing serious evaluation. Clinical trials with human leukocyte interferon in osteogenic sarcoma patients are in progress in Sweden and are being considered in the U.K. and the United States. Preliminary results suggest that, as in the case of chronic hepatitis, short-term interferon treatment delays the metastatic phase of this disease, and that long-term treatment may result in normal life spans.[10,11]

The effects of interferons in vivo are reviewed in detail by Krim and Sanders in Chapter 9.

IV. CONCLUSION

This book reviews the recent advances which have led to the concepts outlined above. Chapters 2, 3, and 4 address themselves to studies with cell A of Figure 1, and to its product(s). Chapter 5, which deals with what is known of the genetics of the interferon system, addresses itself to both cells A and B. Chapters 6, 7, and 8 deal with the effects of interferons in cell B, and Chapter 9 reviews the effects of interferon in vivo.

The interferon system may be viewed as a mirror reflecting the intrinsic beauty of the complex mechanisms that have evolved for the regulation of expression of genetic information on eukaryotic cells. The confusion that currently exists in the area surrounding this field of research is not due to the intrinsically unfathomable nature of the interferon system. It is simply the manifestation of the fact that this system has been examined by scientists with widely varying interests who interpret and discuss their experimental findings within the limits imposed by the rhetorical structures of their particular disciplines. Thus, the virologist describes interferon as an antiviral agent, biochemists refer to it as an inducer of an inhibitor of protein synthesis, the cell biologist calls it a regulator of cell growth, immunologists recognize it as a modulator of immune functions, and the oncologist thinks of it as a possible antitumor agent.

The possibility that the interferon system may lend itself to the study of eukaryotic cell regulatory mechanisms should in itself suffice to justify further sustained efforts towards the complete purification and characterization of the active molecules of interferon preparations, the genetic mapping of their structural and regulatory genes, and the isolation and characterization of the molecules that regulate both the interferon structural genes and those susceptible to activation by interferons. The study of the molecular mechanism underlying the activation and repression of genetic expression which this system may afford can be expected to throw light on fundamental intracellular eukaryotic processes.

Furthermore, since the interferon system involves both inducing and responding cells in a relationship of sequentially and specifically altered metabolic activities, its study is pertinent to that of intercellular relationships. The active molecules contained in interferon preparations may be one or a few words in the language of intercellular communication. Similarly, other words of that language may be components of various other

"preparations," those of lymphokines, chalones, differentiation factors, growth factors, hormones, etc. Some of these mediators have already been purified and even sequenced. The advantage of the interferon system, however, seems to be that interferons lend themselves uniquely (at present) to at least one rapid and precise biological quantification, based on the antiviral activity they induce in susceptible cells. Their very high specific activity in this respect makes it possible to detect amounts of the interferon gene product produced by single cells through the use of exquisitely discriminating and rapid assays.

Finally, the interferon system can be studied in vivo and, hopefully, exploited clinically. It represents a physiologic, reversible set of cellular functions which, under certain circumstances, appear important to survival.

REFERENCES

1. **Ho, M. and Armstrong, J. A.,** Interferon, *Annu. Rev. Microbiol.*, 29, 131, 1975.
2. **DeMaeyer-Guignard, J., DeMaeyer, E., and Montagnier, L.,** Interferon messenger RNA: translation in heterologous cells, *Proc. Natl. Acad. Sci. U.S.A.*, 69, 1203, 1976.
3. **Roberts, W. K., Clemens, M. J., and Kerr, I.,** Interferon-induced inhibition of protein synthesis in L-cell extracts: an ATP-dependent step in the activation of an inhibitor by double-stranded RNA, *Proc. Natl. Acad. Sci. U.S.A., 73:* 3136–3140, 1976.
4. **Lebleu, B., Sen, G. C., Shaila, S., Cabrer, B., and Lengyel, P.,** Interferon, double-stranded RNA, and protein phosphorylation, *Proc. Natl. Acad. Sci. U.S.A.*, 73, 3107, 1976.
5. **Revel, M.,** personal communication, 1976.
6. **Farrell, P. J., Balkow, K., Hunt, T., and Jackson, R. J.,** Phosphorylation of initiation factor IF-E2 and the control of reticulocyte protein synthesis, *Cell*, in press.
7. **Brown, G. E., Lebleu, B., Kawakita, M., Shaila, S., Sen, G. C., and Lengyel, P.,** Increased endonuclease activity in an extract from mouse Ehrlich ascites tumor cells which had been treated with a partially purified interferon preparation: dependence on double-stranded RNA, *Biochem. Biophys. Res. Commun.*, 69, 114, 1976.
8. **Gresser, I., Tovey, M. G., Bandu, M. T., Maury, C., and Brouty-Boye, D.,** personal communication, 1976.
9. **Greenberg, H. B., Pollard, R. B., Lutwick, L. I., Gregory, P. B., Robinson, W. S., and Merigan, T. C.,** Effect of human leukocyte interferon on hepatitis B virus infection in patients with chronic active hepatitis, *N. Engl. J. Med.*, 295, 517, 1976.
10. International Workshop on Interferon in the Treatment of Cancer, Memorial Sloan-Kettering Cancer Center, New York, March 31 to April 2, 1975.
11. **Strander, H.,** personal communication, 1976.