Methods in Enzymology

Volume 78

Interferons

Part A

EDITED BY

Sidney Pestka

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Preface

Progress in interferon research, as in other areas, has reflected advances in the development of new biological and chemical technologies. The methods described in Volumes 78 and 79 of "Interferons" reflect these achievements. By the early 1970s, basic studies on interferon induction allowed the production of small amounts of interferon so that studies on its purification could begin. Of the three classes of interferon (fibroblast, leukocyte, and immune), fibroblast and leukocyte interferons were the focus of attention in many laboratories. The methods for purifying these interferons are described in Volume 78.

With the development of sensitive methods for the assay of amino acids and peptides, the application of high-performance liquid chromatography (HPLC) to protein purification was convenient and feasible, particularly with substances that were available in minute amounts. The purification of interferon by reverse and normal phase HPLC was the first achievement of the purification of proteins by these high-performance procedures. Since then, numerous other proteins have been purified by HPLC with the use of these same techniques. As has been illustrated by additional examples, achievements in interferon research, as in this case, initiated the foundations for achievements in other areas.

Even though some of the interferons were purified, only small amounts were available. However, it was possible to determine their amino acid composition, peptide maps, and some of their amino acid sequences even with picomole to nanomole (0.2 to 20 μg) amounts. The sections in Volume 79 describe these methods.

After the development of assays that could detect the synthesis of biologically active interferons in cell-free extracts and by microinjection directly into intact cells from isolated mRNA, it was feasible to consider cloning the DNA coding for these molecules. The methods leading to and including the construction and identification of DNA recombinants containing the interferon-coding sequences are described in Volume 79.

As a result of the rapid application of these techniques, the complete amino acid sequences of human fibroblast and several leukocyte interferons are now known (see Figs. 1 and 2). Both of these have been expressed in bacteria. Recombinant leukocyte interferon has been purified to homogeneity and has been in clinical trial for about seven months. The isolation of monoclonal antibodies to leukocyte interferon was instrumental in the effective purification of recombinant human leukocyte interferon as well as in the development of convenient immunoassays for the detection of leukocyte interferon. Crystals of recombinant interferon have been obtained and will enable the elucidation of their tertiary structure by X-ray

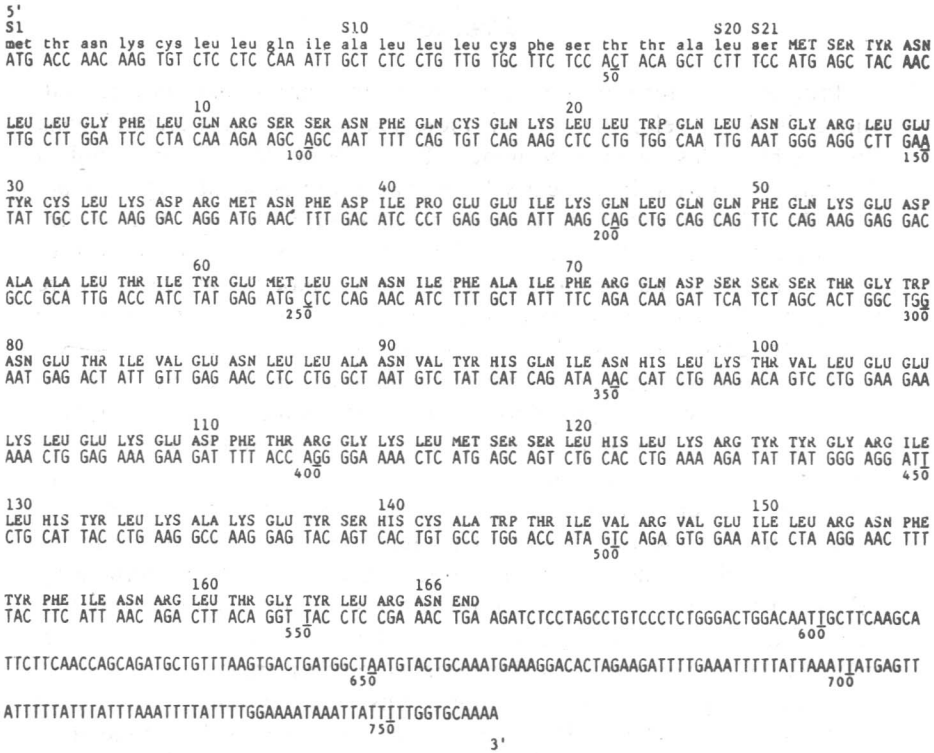

FIG. 1. DNA and amino acid sequence corresponding to recombinant human leukocyte interferon A (IFLrA). See Volume 79 for references.

crystallography. Despite these achievements, much needs to be learned about the biological activity, mode of action, and the clinical efficacy of the interferons. The availability of sufficient amounts for these studies will undoubtedly lead to new insights during this next phase of interferon research. Very much needs to be learned about the "old" interferons, and we will certainly have some additional new ones in the future. The methods in these volumes cover the gamut of these paths.

The contributors to these volumes have spent much time and effort in preparing detailed reports of their methodologies. I am grateful to them all for their many excellent contributions. The staff of Academic Press has been most efficient and supportive throughout this undertaking. Drs. Colowick and Kaplan have provided much sound advice since the time these volumes were initiated. Many colleagues and associates have given me numerous useful suggestions. Special thanks are owed to Sophie Cuber who, with accustomed dedication and thoroughness, provided es-

FIG. 2. DNA and amino acid sequence corresponding to human fibroblast interferon as deduced from the DNA sequence of the gene. See Volume 79 for references.

sential editorial assistance; and to Robert Pestka, for his skill and conscientiousness in preparing the comprehensive Subject Index for this volume.

During the preparation of these volumes, my family has accepted and borne many of my responsibilities in good spirit. Joan has continually sustained an atmosphere for this and other work to be accomplished. Robert, Sharon, and Steven have provided many joys and much good humor vital to me.

SIDNEY PESTKA

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