ADVANCES IN BIOTECHNOLOGICAL PROCESSES

Volume 4

Editors
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Preface

The primary focus of this, the fourth volume in the series **Advances in Biotechnological Processes** is on specific techniques and methodologies employed in the creation, conversion, and application of a diverse array of organic and genetic substances. Among the agents described are human interferons, carcinoembryonic antigen, plant cell suspensions, blood proteins, and sulfate wastes.

The early chapters provide a comprehensive analysis of the isolation, purification, and production of human interferons derived from lymphoblastoid cells. A later chapter, co-authored by the discoverer of eletrofusion, examines the interdisciplinary importance of this new cell hybridization technique, particularly with regard to its interest for physicists.

Carcinoembryonic antigen, a complex immunoreactive glycoprotein, has wide-ranging applications in the detection of various cancers. The production, characterization, and clinical applications of this substance are described comprehensively in the fifth chapter.

The final four chapters in this volume analyze diverse agents derived from human blood, plant cell suspensions, and microbes. One of the problems in biohydrometallurgical processes involves recycling of industrial sulfate wastes. A chapter in this book examines chemical processes for conversion of sulfates to gypsum, and for recycling of wastes by applying sulfate-reducing and sulfur-producing bacteria.

Other interesting subjects, including production of flocculating agents from microbes, methods for improving the levels of products synthesized by plant cells, and fractionation of blood proteins, are also comprehensively reviewed in volume, further enhancing its interdisciplinary appeal.

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Genetic Engineering of Human Interferons From Lymphoblastoid Cells: I. Cloning of Interferon Species Expressed in Sendai-Induced Namalva Cells

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I. INTRODUCTION

Currently three types of interferons (IFN) are recognized on the basis of antigenicity and amino acid sequences—IFN-α, IFN-β, and IFN-γ [1]. The human IFN-α class consists of multiple species, which will be described below. There is some controversy about the number of species of the IFN-β and IFN-γ class. However, only one definitive member of each of these types was isolated and characterized [for review see 2,3].

The ability to induce antiviral activities at femtomole concentrations is a common feature of IFN molecules. In addition, the IFNs exhibit a wide range of activities such as immunomodulatory and antiproliferative activities [4]. Furthermore, various human IFN preparations manifest differences in specific activities on different target cells [see reviews in 3,5,6]. All these differences may be a reflection of the heterogeneity in the IFN species. A systematic study of each interferon species in a given IFN mixture that is produced by a known inducer could lead to an evaluation of future artificial mixtures for clinical purposes. In the next sections we will provide a brief summary of the current state of knowledge regarding the heterogeneity in the human IFN-α gene family and the complex combination of these genes under different inducing conditions in different cells.

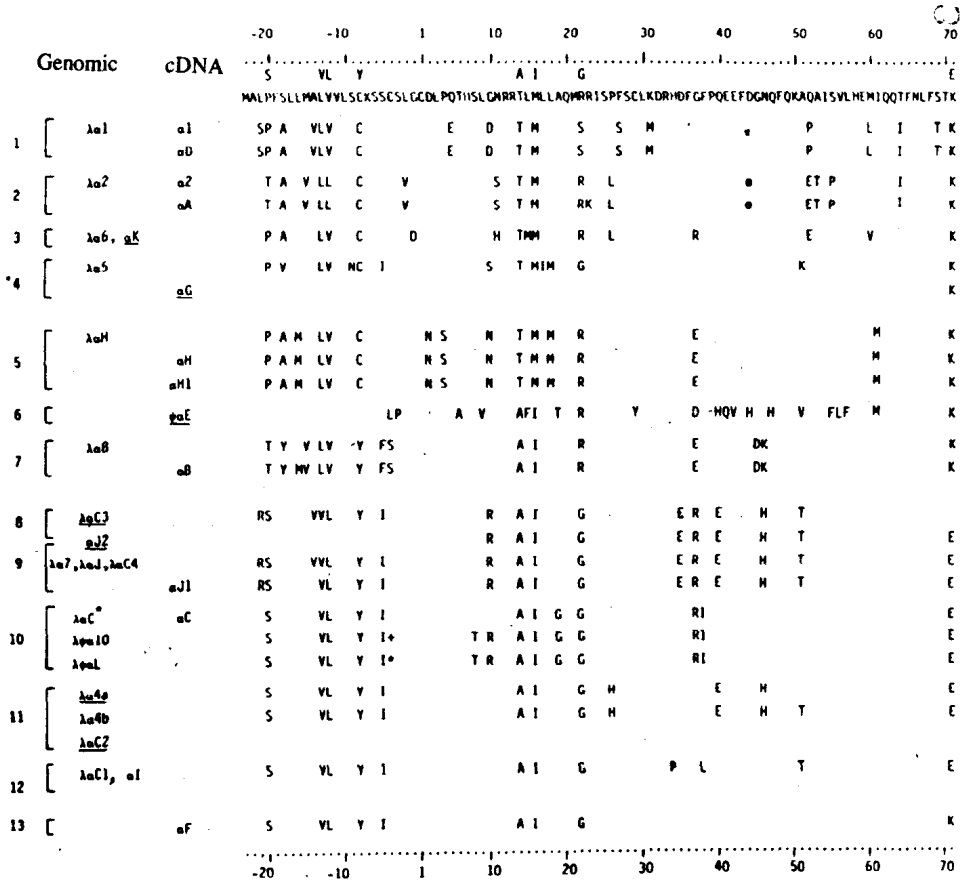
A. The Human IFN-α Family

Thirteen nonallelic genes of the IFN-α gene family have been identified so far by DNA sequencing (Table I). However, the IFNα gene pool is larger, as can be concluded from genomic DNA analysis and from amino acid sequence analysis of IFN-α proteins. Analysis of two genomic DNA libraries [22,23] revealed more than 20 IFN-α genes [2,3, 7–12]. Some of these genes are distantly related (designated IFN-α-like genes) showing less than 80% nucleotide sequence homology to IFN-α1 [10] or IFN-αC [3]. Amino acid sequence analysis of IFN-α polypeptides was performed on IFN-α mixtures or on individual IFN-α polypeptides prepared from different cell types [see

reviews in 19–21]. Collins [2] has compiled and compared the amino acid sequences of IFN-α polypeptides and amino acid sequences deduced from IFN-α genes or cDNAs. From this comparison it seems that 19 polymorphisms appear in the peptide sequences for which no known sequenced gene exists. This may be due to either artifacts in the amino acid sequencing method or to high incidence of allelic variants as shown in Table I. That a high incidence of allelic variation does indeed exist is clearly shown by the isolation of four alleles from a genomic library of a single individual [22]. These four alleles (α4a, α4b, α10, and αL) represent two loci [10–12]. In a genomic library from a different individual [23] an additional allele for each of the two loci was found [3] (Table I). The polymorphism is also manifested by the cases where a large number of gene sequence polymorphisms exist for which no corresponding amino acid has been found in the peptide sequences [2]. This may be a result of (a) use of inducing conditions that are not suitable for expression of the given gene (b) difficulties in detection of the specific IFN polypeptide that is expressed at low efficiency, (c) the presence of pseudogenes that either express defective polypeptides (like IFN-αL [11], IFN-α10 [10], and IFN-αE [13]), or which are not induced at all.

Weissmann et al. [12] proposed to classify the IFN-α into two main subfamilies. Subfamily I includes α1(D), α2(A), α5(G), and α6. Subfamily II, previously designated subfamily C [11], consists of α10(C), α7, α4, αC1, and αF. An intermediate subfamily includes αH and α8. All of the IFN-α genes and IFN-β1 are localized on chromosome 9 [24]. The genes of subfamily I are dispersed, each one of them was found on a different 15–20-kilobase (kb) DNA genomic segment [7,9,12]. Subfamily C genes are more clustered. A cluster of three genes was found on a 20-kb DNA segment [3,11,12]: αC (α10, αL, or αC* allele), αJ (α7, αJ, or αC4 allele), and α4 (α4a, α4b, or αC2 allele). Another DNA segment contains αC1 gene as well as αH and another H-like gene [8,68]. αC, αJ, and α4 show 92–96% amino acid sequence homology (Table I) and are flanked by regions of extensive homology [10]. Nucleotide sequencing of about a 10-kb DNA segment that includes two of these genes shows that αJ and αL(αC) are located on two tandem repeats of 4 kb showing an extremely high degree of homology [11]. This arrangement probably reflects a recent gene duplication event. The minimal divergency among these genes can be useful in studying the response of the different but closely related genes to various inducers, and in elucidating the DNA sequences responsible for the gene expression as well as in the determination of structure-function significant domains. Such a comparison between the antiviral activity of IFN-αJ and IFN-αC will be described in the following chapter [6].

TABLE I. Amino Acid Sequences of Human α-Interferons*



The amino acid (a.a.) sequences were deduced from nucleotide sequences of cloned genomic DNAs and cDNAs. The consensus a.a. sequence is adapted from Weissmann et al. [12]. Differences from this sequence are indicated for each gene, λα1 [9], α1 [14], λα2 [7], α2 [15], λα4–λα10 [12], αA–αH1 [13], λαC1 (=αI), λαH [8,69], λαL, λαJ [11], αJ1 [16,18], αJ2, αK [17]. All the genes designated by λ are derived from a genomic library of one individual [22], except λαC, λαC2, λαC3, λαC4 [3] which are derived from a different individual [23]. The IFN-α species that are underlined have incomplete sequences: αG lacks the sequence from a.a. −23 to 33; α4a from a.a. 126 to 161; αE from a.a. −23 to −5; αC2 from a.a. −23 to 77; αC3 from a.a. 54 to 166; αK and αJ2 lack the signal peptide. The basic organization of the table is according to Weissmann et al. [12], which is now updated to include nine additional IFN-α species. Genomic sequences for the same locus are displayed consecutively and are indicated by numbers (on the left side). cDNA sequences which did not