FORTY YEARS OF INTERFERON

P. KONTSEK[1], E. KONTSEKOVÁ[2]

[1]Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 46 Bratislava; [2]Department of Microbiology and Virology, Comenius University, Bratislava, Slovak Republic

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Summary. – The nature and mode of action of interferon (IFN) have been intensively studied for more than 40 years. In this review, we summarize the current knowledge of IFN putting emphasize on transitions in its definition, understanding of its physiological role and nomenclature, and on brief characterization of individual IFN families. Finally, the evolution of IFN and relations between the IFN families are discussed.

Key words: interferon; definition; nomenclature; physiological role; evolution

From antiviral protein to pleiotropic cytokine

Since the 1930's, the viral interference has been a well known phenomenon in virology. Viral interference means that infection with an avirulent or inactivated virus protects cells from subsequent infection with live or (more) virulent virus. During studies on viral interference in the 1950's, two groups of authors have independently reported that the interfering virus induced a soluble substance that made other cells resistant to subsequent viral infection. Discovered as a virus interference factor in 1954 by Nagano and Kojima in rabbits (Nagano and Kojima, 1954), this substance has became much more popular under the name "interferon" (IFN) – a term coined for a similar antiviral agent produced by chicken cells by Isaacs and Lindenmann three years later (Isaacs and Lindenmann, 1957). Ever since the discovery, IFN presented both a promise and a challenge to scientists in various fields of biomedical research. During ensuing years, it became clear that the term "IFN" covers a group of proteins with a broad spectrum of effects, and that the antiviral activity is only one of many of IFN functions. Until 1980, most experiments with IFN have been performed with a material of which less than 1% was true IFN. Achievements in the field of molecular biology in the early 80's led to a qualitative shift from the study of IFN as an antiviral factor whose existence was regarded by many with considerable scepticism to the study of IFN as exactly defined molecules with known amino acid sequence.

IFNs are a family of inducible (glyco)proteins produced by vertebrate cells (fishes, reptiles, birds and mammals, except amphibia) to different biologic stimuli (Borecký, 1983; DeMaeyer and DeMaeyer-Guignard, 1988). The ability to induce IFN is not an exclusive property of viruses, IFN-inducers include also bacteria, mycoplasma and protozoa, certain cytokines, mitogens, natural and synthetic double-stranded RNA and other substances (Borecký, 1983). Most IFNs are glycoproteins, however, their sugar moiety has not an apparent structural or functional role (Kontsek, 1994). Apart from their antiviral activity, by which they were discovered, IFNs exert also other activities including inhibition of cell growth, antitumour action, effects on cellular differentiation and a wide range of immunomodulatory effects. IFNs are not the effector molecules in their own right. They confer pleiotropic activities after binding to specific receptors on the surface of target cells, which are coupled to intracellular signal transduction and second messenger pathways (Borecký, 1983; Pestka *et al*., 1987; DeMaeyer and DeMaeyer-Guignard, 1988). A receptor to nucleus signal transduction by IFNs involves the activation of tyrosine kinases of the Janus kinase (JAK) family and the phosphorylation of latent cytoplasmic transciption factors – STAT proteins (the signal transducers and activators of transcription) that dimerise and translocate from the membrane to

Abbreviations: 3D = three-dimensional; IFN = interferon; JAK = Janus kinase; STAT = signal transducers and activators of transcription

the nucleus and effect the transciptional activation of specific target genes (Darnell *et al*., 1994). New proteins produced after signal transduction confer upon the cell those activities we ascribe to IFNs.

The definition of IFN has evolved in association with transition from biological and physico-chemical criteria to recent molecular-biological ones. In 1973 (Lockart, 1973), seven criteria were suggested for use in classifying an antiviral substance as IFN. In 1980, these criteria were condensed into a brief definition of IFN as a factor of protein nature which exerts virus-unspecific antiviral activity at least in homologous cells through cellular metabolic processes involving RNA and protein syntheses (Stewart *et al*., 1980). Later, the biological attitude was stepping aside and the most important criterium for classifying a protein as an IFN became the nucleotide and amino acid sequence of the respective gene and its protein product, respectively. From the functional point of view, IFNs together with interleukins, chemokines and growth factors are now regarded as cytokines. Cytokines are secretory regulatory proteins or glycoproteins, which act in organisms as chemical communicators between cells and are involved in a number of important physiological processes (Callard and Gearing, 1994). A recent brief definition of IFN is following: IFNs are pleiotropic cytokines possessing antiviral, antiproliferative and immunomodulatory activities. At present, special attention is paid to regulatory functions of IFNs in association with their hormon-like effects. IFN is considered an important mediator of cellular communication between neuroendocrine and immune systems (Borecký, 1992). This led to a reevaluation of the physiological role of IFN and to a more complex understanding of its position in organism: IFNs belong to a network of regulatory cytokines that are all involved in the homeostatic control of cell function and replication under normal conditions, and that become active participants in host cell defense when an emergence arises because of infection (De Maeyer and DeMaeyer-Guignard, 1988).

Nomenclature of IFNs

IFNs were initially classified based on cell types from which they were derived, their chemical properties and their antigenicity (Stewart, 1979). Three types of IFNs were recognized: leukocyte (alpha), fibroblast (beta) and immune (gamma). Whereas IFNs alpha and beta were acid-stable, IFN-gamma was acid-labile. Such classification served the purpose until the 1980's. With the cloning of IFN molecules this nomenclature has also evolved, recently being based primarily on the structure of IFN genes and proteins. In mammals, there are six IFN families designated alpha, beta, gamma, delta, omega and tau. These IFNs have been grouped into two separate classes: type I and II. The type I IFN comprises IFN families alpha, beta, omega, tau and delta, the type II IFN contains IFN-gamma only. These two classes represent distinct structures of alpha-helical globular proteins (Figs. 1,2) recognized by distinct multicomponent cellular receptors for type I and type II IFNs (Zavyalov *et al*., 1989; Farrar and Schreiber, 1993; Uzé *et al*., 1995).

Characterization of IFN families

Type I IFNs represent a heterogeneous group of polypeptides sharing sequence homology. They are divided into five families: alpha, beta, omega, tau and delta, based primarily on divergence in nucleotide sequence and antigenic distinctions. The number of genes and polypeptides in each family varies from species to species. Whereas non-mammalian vertebrates (fish and amphibia) contain only IFN-beta genes, other vertebrates contain both IFN-alpha and -beta genes (Wilson *et al*., 1983). Type I IFNs are encoded by a set of clustered intronless genes, a fact which is rare for eukaryotic nuclear genes. In addition to the identical three-dimensional (3D) structure (Zavyalov *et al*., 1989; Senda *et al*. 1995), all the type I IFNs share the stability at pH 2, bind to the same cellular receptor, are inducible by viruses, and their functional unit is a monomer. In the JAK-STAT signalling pathway, type I IFNs induce the phosphorylation and activation of the tyrosin kinases tyk-2 and Jak-1.

IFN-alpha is represented by a family of related polypeptides. They are produced after virus-induction especially by peripheral blood leukocytes and haematopoetic cell lines. IFN-alpha was first cloned by Nagata *et al*. in 1980 (Nagata *et al.,* 1980). Multigene families of IFN-alpha have been detected in all mammalian genomes examined to date (Pestka *et al*., 1987).

IFN-beta is historically the first discovered and first cloned (Taniguchi *et al*., 1979) type of IFN. The IFN-beta family is represented by a single gene in many species including man. There are also species with two (horse, lion, rabbit) or even more (swine, cattle) IFN-beta genes. Natural producers of IFN-beta are fibroblasts induced with viruses or double-stranded RNA. IFN-beta seems to be more species-specific than IFN-alpha (DeMaeyer and DeMaeyer-Guignard, 1988).

*IFN-omeg*a was described independently by three groups of authors in 1985 (Capon *et al.*, 1985; Feinstein *et al*., 1985; Hauptman and Swetly, 1985). IFN-omega is widely distributed among mammals. It constitutes multiple genes in cattle, horse and human, but apparently was deleted from the canine genome (Adolf *et al*., 1990). Functional IFN-omega protein consisting of 172 amino acids is a little bit longer than

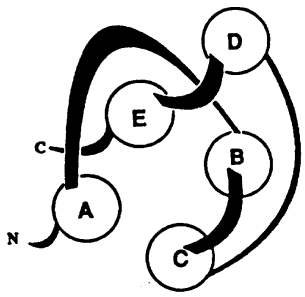

Fig. 1
Structure of type I IFN (monomer)
Five alpha helices (A-E) are connected by loops. N- and C-termini are indicated. Adapted from Mitsui *et al.* (1993).

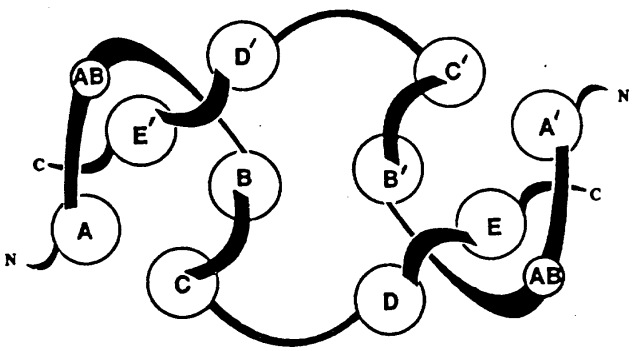

Fig. 2
Structure of type II IFN (homodimer)
Twelve alpha helices (A-E, A'-E', AB (2x)) are connected by loops. N- and C-termini are indicated. Adapted from Mitsui *et al.* (1993).

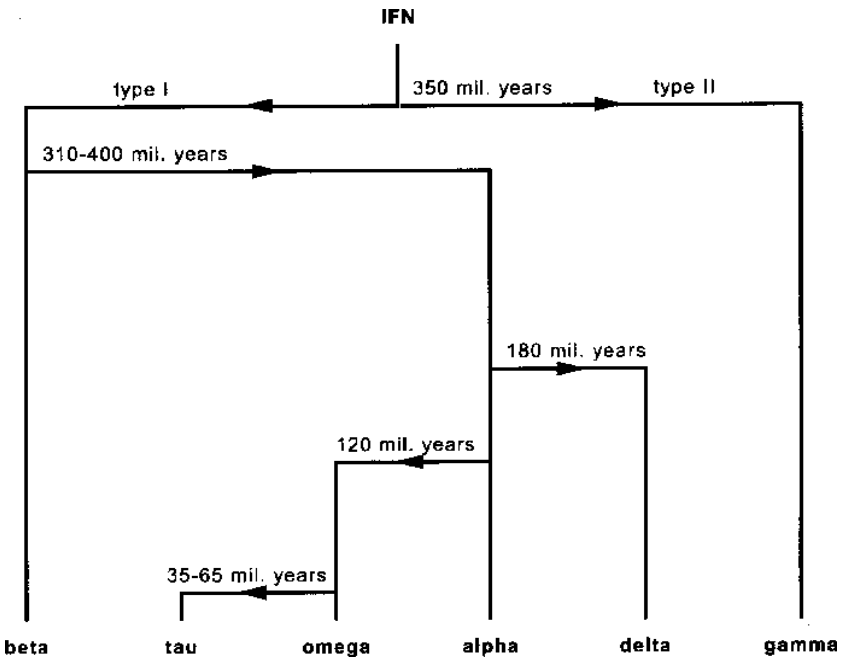

Fig. 3
Phylogenetic tree of IFN
Estimated times of branch points in millions (mil.) of years.

166-residue-long IFN-alpha/beta polypeptides. IFN-omega is predominantly secreted by the same cells as IFN-alpha.

IFN-tau has been known as ovine trophoblast protein until Imakawa *et al*. (1987) proved its relation to the type I IFNs. This 172-amino-acid-long polypeptide exhibits the highest similarity to IFN-omega. IFN-tau is not readily inducible by virus and is produced by the trophoblast of the conceptus during a short period close to implantation. It is the first type I IFN whose biological role was not primarily antiviral. IFN-tau is expressed from a multigene family which is restricted to ruminant ungulate species (e.g. cattle, ovine, goat) (Leaman and Roberts, 1992).

IFN-delta was discovered only recently in pigs by Lefevre and Boulay (1993). This IFN is produced by the trophoblast during the period of implantation in uterus. The mature polypeptide is 149 amino acids in length and represents smallest type I IFN. IFN-delta is derived from a multigene family containing only a single funtional gene. In other mammalian species, IFN-delta has not been detected yet.

Type II IFNs comprise the only species, IFN-gamma, which was discovered by Wheelock in 1965 (Wheelock, 1995). In contrast to the type I IFNs, IFN-gamma is acid-labile and is inactivated at pH 2 in few minutes. Its induction occurs during immune reactions in mitogen- or antigen-stimulated T or natural killer cells. Of particular importance are the immunomodulatory effects of the IFN-gamma, which is one of the few physiological inducers of the histocompatibility class II antigen (Billiau, 1996). The effect of IFN-gamma is highly species-specific and is mediated via the type II IFN receptor. In the JAK-STAT signalling pathway, IFN-gamma induces phosphorylation and activation of Jak-1 and Jak-2. IFN-gamma was cloned in 1982 (Gray *et al*., 1982; Devos *et al*., 1982). Only a single gene for IFN-gamma which contains three introns and codes for polypeptides shorter than other type I IFNs has been detected in mammalian and avian species. The functional unit of IFN-gamma is a homodimer and its 3D structure was solved by Ealick *et al*. in 1991 (Ealick *et al.*, 1991).

Evolution of IFN

The type I IFNs possibly evolved from a common primordial progenitor gene - ancestral IFN-beta - by gene duplication. It has been estimated that the split of IFN-alpha/beta genes could have happened just before the mammals-birds/reptiles divergence about 310-400 million years ago (Miyata *et al*., 1985; DeMaeyer and DeMaeyer-Guignard, 1988) (Fig. 3). Since several mammals including man contain multiple IFN-alpha gene loci, it appears that the multiple loci had been established before the mammalian radiation (85 million years ago), whereas the origin of their sequences is new and goes back to a single ancestor about 40 million years ago (Miyata *et al*., 1985). As the next evolutionary oldest type is considered IFN-delta, whose ancestral sequence diverged from IFN-alpha gene about 180 million years ago, while IFN-omega and IFN-alpha genes diverged about 120 million years ago (Capon *et al*., 1985; Lefévre and Boulay, 1993). The IFN-tau genes evolved from virus-inducible IFN-omega within the last 30-65 million years. Their most recent divergence could explain why their existence in mammals is limited to ruminants (Leaman and Roberts, 1992). Each class of type I IFN is encoded by a multigene family, but the reason for the evolutionary selection of such gene families is largely unknown.

Existence of the avian IFN-gamma indicates that the emergence of the two principal types of IFN predated the divergence of birds and mammals that had occured some 350 million years ago (Digby and Lowenthal, 1995). Nevertheless, an evidence for a common ancestral gene for type I and II IFNs still lacks, and their evolutionary relationship remains unclear. IFN-gamma shows no apparent homology in amino acid sequence to type I IFNs. Although partial structural similarity between human IFN-gamma and human IFN-alpha/beta was already reported (DeGrado *et al*., 1982), their sequence alignment on the basis of predicted secondary structure does not indicate a significant homology. However, comparison of 3D structures of type I and II IFNs reveals some similarity in folding topology of polypeptides (Ealick *et al.,* 1991). At present, the most significant evidence on the possible evolutionary relationship between type I and type II IFNs comes from the structural homology between their receptors (Bazan, 1990) and existence of the shared components used in the signal transduction pathways of type I and type II IFNs (Vilček and Oliveira, 1994).

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BOOK REVIEW

Oncogenes as Transcriptional Regulators

M. Yaniv, J. Ghysdael (Eds): Oncogenes as Transcriptional Regulators. Vol. 1: Retroviral Oncogenes. Vol. 2: Cell Cycle Regulators and Chromosomal Translocation. Birkäuser Verlag, Basel - Boston - Berlin, 1997, pp. 249 (Vol. 1), 162 (Vol. 2).

Study of a transcriptional regulation of the gene expression has been one of the basic approaches leading in the last decade to a better understanding of biological role of many genes. To become ready to undertake such a study in newly identified experimental systems, scientists have not only to be able to use appropriate methods, but also to be aware of a diversity of molecular mechanisms taking place during the processes of transcriptional activation and repression and of a variety of components participating in transcriptional machinery.

Many transcriptional regulators play an important role in molecular pathways that control cell proliferation, differentiation and transformation. They are encoded by genes defined either as oncogenes or as tumour suppressor genes. These genes and their products attract a lot of attention of scientific community due to their crucial position in cellular processes. Currently published monograph entitled "Oncogenes as Transcriptional Regulators", edited by M. Karin within a series "Progress in Gene Expression", represents a valuable source of many data on structure, specificity of DNA binding, and function of target genes regulated by transcriptional factors derived from viral and cellular oncogenes and tumour suppressor genes. In addition to these data, the book contains many schematic illustrations for an easier understanding of described regulatory pathways and a large list of relevant literature sources.

The monograph is divided into two volumes. The first one consists of six chapters describing classical retroviral oncogenes whose discovery was the first step to identification of their cellular counterparts. The subjects of discussion are products of *myc*, *myb*, *ets*, *erbA*, *rel*, *fos* and *jun*. These transcriptional factors are activated upon mitogenic stimulation and their oncogenic activity is frequently linked to a block of differentiation. The team of authors that is composed of renowned specialists in the field paid attention not only to transcriptional activation and/or repression mediated by these proteins, but also to signalling pathways leading to their oncogenic conversion, to functional interactions of oncoproteins, their cross-talk with other transcriptional factors, and to their non-transformational functions, e.g. role of Myc in apoptosis.

The second volume is dealing with regulators of cell cycle and products of tumour suppressor genes, namely EF2 transcriptional factors, Rb protein family and p53 protein. Furthermore, the volume contains two chapters reviewing consequences of chromosomal translocations that result in chimeric proteins with aberrant or dual functions. Finally, the editors decided to include a chapter on EBNA2, a viral protein regulating transcription of both viral and cellular genes that is essential for the immortalisation of human B lymphocytes by the Epstein-Barr virus.

The authors of the monograph offer a complex picture built up of up-to-date knowledge of the biological processes studied at the level of transcriptional regulation. A detailed analysis of data and intelligible text makes this book suitable for both experienced investigators and those who are recently entering the fields of molecular and cellular biology as well as related areas of research.

S. Pastoreková, Bratislava