25. Interferons Alpha, Beta, and Omega

Anthony Meager

1. Introduction

1.1 OUTLINE OF DISCOVERY AND CHARACTERIZATION OF INTERFERONS (IFN)

The phenomenon of viral interference was first described nearly 60 years ago when Hoskins (1935) described the protective action of a neurotropic yellow fever virus against a viserotropic strain of the same virus in monkeys. Although viral interference was further investigated in the 1940s and 1950s, the underlying mechanism was not discovered until 1957 when Isaacs and Lindemann, working at The National Institute for Medical Research (London, UK), isolated a biologically active substance from virally-infected chicken cell cultures that, on transfer to fresh chicken cell cultures,

produced a protective antiviral effect (Isaacs and Lindemann, 1957). The word Interferon (IFN) was coined for this substance. Its discovery aroused considerable scientific and medical interest since by 1957 antibiotics were widely available to control bacterial infections, but, in stark contrast, viral diseases such as influenza, measles, polio, and smallpox were virtually untreatable. Interest was further heightened by many subsequent studies that demonstrated that IFN could be produced by human cells and was active against a broad spectrum of viruses (see Schlesinger, 1959, for an early review).

At that time, IFN was being hailed by the media as a wonder drug, but it soon became clear that IFN was being produced naturally in too small quantities for that extravagant claim to be immediately confirmed. In fact, the low production of IFN was to bedevil attempts both to characterize it molecularly and evaluate it clinically for many years following its discovery.

Although the protein nature of IFN was recognized at an early stage in its development (see Fantes, 1966, for an early review), it was only following the introduction of large-scale production methods in the 1970s (Cantell and Hirvonen, 1977) and the simultaneous development of efficient purification procedures (Knight, 1976; Rubinstein et al., 1978) that sufficient amounts of partially pure IFN protein became available for characterization and clinical use. Gradually, it became apparent that IFN was not a single protein and that there were likely to be different types of IFN molecules. However, despite progress in the area of purification and in initial characterization by sequencing N-terminal polypeptides, IFN proteins all but defied full characterization until the advent of recombinant DNA (rDNA) technology in the late 1970s. This technology, spurred on by the pharmaceutical industry's desire to produce pharmacologically active proteins cheaply, revealed that one type of human IFN, now designated IFN-α, was a mixture of several closely related proteins, termed subtypes, expressed from distinct chromosomal genes (Nagata et al., 1980). Second and third types of IFN, designated IFN-β and IFN-γ respectively, have subsequently been "cloned" (Taniguchi et al., 1980; Gray et al., 1982) but, unlike IFN-α, are single protein species. IFN-β is molecularly related to IFN-α subtypes but is antigenically distinct from them, whereas IFN-γ is both molecularly and antigenically distinct from either IFN-α subtypes or IFN-β. (For this reason, IFN-γ is considered separately elsewhere in this volume.) Finally, a fourth type of IFN, antigenically distinct from IFN-α and IFN-β, but molecularly related to both, has more recently been cloned and characterized. Rather untypically, this new IFN type has been designated IFN omega (IFN-ω) (Adolf, 1987).

The genes for IFN-α subtypes, IFN-β and IFN-ω are tandemly arranged on the short arm of chromosome 9.

They are only transiently expressed following induction by a variety of exogenous stimuli, including viruses. IFN-α, IFN-β and IFN-ω proteins are synthesized from their respective mRNAs for relatively short periods following gene activation and are secreted to act, via specific cell surface receptors, on other cells. Early studies on the characterization of IFN receptors indicated that IFN-α and IFN-β were likely to share a common receptor, but it has only been comparatively recently that such receptors have been cloned (Uzé et al., 1990; Novick et al., 1994).

IFN actions are initiated by activated receptors and cytoplasmic signal transduction pathways, which are now well characterized for the IFN-α/β receptor, and manifested following expression of a number of IFN-specific inducible genes. Induction of the antiviral state, which is dependent on such protein synthesis, may now be viewed as just one of the many activities attributed to IFN in general; these activities include inhibition of cell proliferation and immunomodulation (see Pestka et al., 1987, for a review).

1.2   NOMENCLATURE

In the 1960s, two types of IFN were defined on the basis of the capacity of their antiviral activity to withstand acidification to pH 2. These were termed type I IFN for acid-stable IFN and type II IFN for acid-labile IFN. Type I IFN included IFN produced by virally infected leukocytes, alternatively known as leukocyte IFN, and IFN produced by virally infected human diploid fibroblasts, alternatively known as fibroblast IFN. Type II IFN, which was only produced by antigenically or mitogenically stimulated human peripheral blood mononuclear cells (PBMC), has often been referred to as immune IFN (Stewart, 1979).

Antigenic differences were described for leukocyte and fibroblast IFN and these were put on a molecular basis when knowledge of their respective N-terminal amino acid sequences became available (Allen and Fantes, 1980; Knight et al., 1980; Levy et al., 1980; Zoon et al., 1980). At that time, an international nomenclature committee (Stewart et al., 1980) reviewed the growing evidence for the existence of distinct molecular forms of IFN and introduced the Greek alphabetical system to apply to the then known antigenically distinct types of IFN. Leukocyte IFN was designated IFN-α, fibroblast IFN was designated IFN-β, and immune IFN became IFN-γ. However, complications immediately arose when it was revealed, following the cloning of several different leukocyte IFN complementary DNAs (cDNAs) (Nagata et al., 1980; Brack et al., 1981; Goeddel et al., 1981; Streuli et al., 1980), that leukocyte IFN was heterogeneous and contained many different, molecularly and antigenically related species, now commonly referred to as subtypes. The research group at

Hoffmann-La Roche labeled the subtypes produced in *E. coli* αA, αB, αC, αD, etc. (Evinger *et al.*, 1981; Rehberg *et al.*, 1982), distinguishing them from natural components of leukocyte IFN (Rubinstein *et al.*, 1981), while the Biogen group labeled these recombinant subtypes α1, α2, α3, α4, etc. (Streuli *et al.*, 1980), regrettably without an appropriate alphabetical–numerical correspondence: for example, αA ≡ α2, αD ≡ α1. However, the numerical system now prevails. When an IFN preparation is a mixture of IFN-α subtypes, e.g., leukocyte IFN, lymphoblastoid IFN, this is often designated IFN-αn.

The later cloning of cDNAs encoding IFN-α-like proteins (Capon *et al.*, 1985; Hauptmann and Swetly, 1985) initially led to the naming of this new IFN as IFN-α subclass II, with all of the earlier-characterized IFN-α subtypes being reclassified as IFN-α subclass I. This large, unwieldy nomenclature system has been superseded by the renaming of IFN-α II as IFN-ω; this has been generally accepted with the finding that IFN-ω is antigenically distinct from IFN-α and IFN-β proteins and thus qualifies as a separate type of IFN (Adolf, 1987).

Fortunately, the nomenclature for IFN-β has remained straightforward since there is only one protein species, at least in humans (Derynck *et al.*, 1980, 1981; Taniguchi *et al.*, 1980).

From the initial cloning of IFN-α cDNAs, there has been a plethora of reports on the cloning of new, and sometimes distinct, genomic and cDNA clones, and fairly disparate nomenclatures have arisen. Diaz and Allen (1993) therefore undertook the considerable task of compiling the IFN genes and genomic and cDNA clones from the literature and introduced an arabic-alphabetical system for naming IFN genes to enable their distinction from IFN proteins. Thus, IFN-α genes became IFNA genes with the addition of a numeral to denote subtype, i.e., IFNA1, IFNA2, etc. (Table 25.1). The IFN-β gene became IFNB and, since there is only one gene in humans, it is referred to as IFNB1. For IFN-ω genes, W has been used; hence IFNW1 (Table 25.1). Besides genes that are capable of being expressed and translated into IFN proteins, there are a number of pseudogenes which are unable to give rise to IFN proteins, and which in this new nomenclature system are designated by a P, e.g., IFNAP22, IFNWP2, or simply IFNP1 where pseudogenes are clearly IFN-like but cannot be definitely included in any one of the IFNA, IFNB or IFNW gene families (Table 25.1).

The IFN genomic and cDNA clones have been designated in a variety of ways, as illustrated in Table 25.1. Pseudogenic or non-translatable clones are normally prefixed with a Greek ψ. For the purposes of this chapter, and to reduce the complexity of naming IFN clones and proteins, the nomenclature system adopted by Weissmann and colleagues will be adhered to: IFN-α₁, α₂, α₄, α₅, etc.

2. IFN Genes

2.1 NUMBERS, STRUCTURE, AND LOCALIZATION

In humans, there are 14 nonallelic IFNA genes, one of which, IFNAP22, is a pseudogene (Table 25.1). In addition, there are a further four nonallelic pseudogenes that possibly also belong to the IFNA gene family. Probable allelic variants of certain IFNA genes, e.g., IFNA2, are also known to exist (Streuli *et al.*, 1980; Goeddel *et al.*, 1981; Dworkin-Rastl *et al.*, 1982; Emanuel and Pestka, 1993). This extensive family of IFNA genes are tandemly arranged on the short-arm of chromosome 9 (9p23) and span a region of approximately 400 kb (Owerbach *et al.*, 1981; Shows *et al.*, 1982; Slate *et al.*, 1982; Ullrich *et al.*, 1982). The IFNB gene and the IFNW gene/pseudogene family are also located in the same region of chromosome 9 (Meager *et al.*, 1979a,b; Owerbach *et al.*, 1981; Henry *et al.*, 1984; Capon *et al.*, 1985).

There is a high degree of homology among the IFNA genes, but these show much less homology to either IFNB or IFNW genes. Nevertheless, all of these IFN genes share the common feature of being intron-less (Taniguchi *et al.*, 1980; Goeddel *et al.*, 1981; Houghton *et al.*, 1981; Capon *et al.*, 1985), suggesting a very ancient origin of their common ancestral gene. It has been proposed that the primordial IFN gene arose some 500 million years ago, with the first split occurring around 400 million years ago to yield the first IFNA and IFNB genes (Wilson *et al.*, 1983). Since then the IFNA gene has evolved and duplicated many times to give rise to the multiple IFNA genes found in present-day animals and man (Mijàta and Hayashida, 1982; Gillespie and Carter, 1983). Around 100 million years ago, an IFNA gene appears to have diverged sufficiently from the main group to give rise to the IFNW gene family, which is present in most mammals except mice and dogs (Himmler *et al.*, 1987; Roberts *et al.*, 1992).

It is not clear what characteristic of IFNA and IFNW genes enabled the numerous reduplication events to occur in comparison to the nonexistent or more limited (some mammals have more than one IFNB gene, e.g., bovines (Wilson *et al.*, 1983)) reduplication of the IFNB gene (Ohlsson *et al.*, 1985). It is apparent that gene conversion, as a result of mismatch repair and unequal crossover, contributed significantly to the creation of distinct, but highly homologous, nonallelic IFNA (and IFNW) genes (De Maeyer and De Maeyer-Guignard, 1988). In most cases, both coding and noncoding regions have diverged, but in the case of IFNA13 the coding region has remained identical to that of IFNA1, although its 5′ and 3′ flanking regions have diverged (Todokoro *et al.*, 1984).

The structures of IFNA, IFNB, and IFNW genes are similar. Each gene has a 5′ regulatory promoter region upstream from the transcriptional start (cap) site, a

Table 25.1   Nomenclature of the human interferon genes and proteins

IFN genes; new symbols	Corresponding genomic clones (examples)	Corresponding cDNA clones (examples)	Corresponding proteins
IFN-α			
IFNA$_1$	IFN-α_1	IFN-α_1, LeIF-D	IFN-α_1 (D)
IFNA$_2$	λa$_2$, IFN-A	IFN-α_2, LeIF-A	IFN-α_2 (A)
IFNA$_4$	IFN-α_{4a}, IFN-α_{7b}	–	IFN-α_4
IFNA$_5$	IFN-α_5, IFN-α_{61}	LeIF-G	IFN-α_5 (G)
IFNA$_6$	IFN-α_6, LeIF-K	–	IFN-α_6 (K)
IFNA$_7$	IFN-α_7, LeIF-J	IFN-α_{J1}	IFN-α_7 (J1)
IFNA$_8$	IFN-α_8, IFNα_{B2}	LeIF-B, IFN-B	IFN-α_8 (B)
IFNA$_{10}$	ψIFN-α_{10}, ψLeIF-L	LeIF-C	IFN-α_{10} (L)
IFNA$_{13}$	IFN-α_{13} (similar to IFN-α_1)	–	IFN-α_{13}
IFNA$_{14}$	IFN-α_{14}, IFN-α_N	LeIF-H	IFN-α_{14} (H1)
IFNA$_{16}$	IFN-α_{16}, IFN-α_{WA}	IFN-αN (Gren)	IFN-α_{16} (WA)
IFNA$_{17}$	IFN-α_{17}, IFN-α_T	IFN-α_1, IFN-α_{88}	IFN-α_{17}
IFNA$_{21}$	IFN-α_{21}	LeIF-F	IFN-α_{21} (F)
IFNAP$_{22}$	ψIFN-α_{22}	ψLeIF-E	IFN-α_{22} (E)
IFN-β			
IFNB$_1$	IFN-β	IFN-β	IFN-β
IFN-ω			
IFNW$_1$	IFN-α_{II1}	IFN-αII$_1$, IFN-ω	IFN-ω
IFNWP$_2$	ψIFN-α_{II2}	–	–
IFNWP$_4$	ψIFN-α_{II4}	–	–
IFNWP$_5$	ψIFN-$\alpha_{\omega5}$	–	–
IFNWP$_9$	ψIFN-α_{II9}	–	–
IFNWP$_{15}$	ψIFN-α_{II3} or 15	–	–
IFNWP$_{18}$	ψIFN-α_{IIM} or 18	–	–
IFNWP$_{19}$	ψIFN-α_{19}	–	–
IFN pseudogenes not included in α or ω gene families			
IFNP$_{11}$	ψIFN-α_{11}	–	–
IFNP$_{12}$	ψIFN-α_{12}	–	–
IFNP$_{20}$	ψIFN-α_{20}	–	–
IFNP$_{23}$	ψIFN-α? (closely linked to IFNA1)	–	–

Adapted and modified from Diaz and Allen (1993) and Allen and Diaz (1996), which see for specific references for genomic clones and cDNAs. Reproduced with permission of Mary Ann Liebert, Inc., New York, USA.

coding region containing a nucleotide sequence encoding a signal polypeptide of 21–23 mainly hydrophobic amino acids, which is typical for secreted proteins, and consecutively the sequence encoding the mature IFN protein, followed by the 3′ flanking noncoding region, which can vary in length up to 450 base pairs (bp) (Figure 25.1) (Derynck et al., 1980, 1981; Nagata et al., 1980; Streuli et al., 1980; Taniguchi et al., 1980; Degrave et al., 1981; Goeddel et al., 1981; Gross et al., 1981; Lawn et al., 1981a,b; Gren et al., 1984; Capon et al., 1985; Henco et al., 1985). The 5′ flanking region contains a TATA or Hogness box, which delineates the boundary of the upstream promoter, approximately 30 bp from the cap site. Farther upstream are found a number of hexameric repeat sequences GAAANN, where N can be any base, which in their dimeric or multimeric forms act as binding sites for nuclear transcription factors and repressor molecules (Fujita et al., 1985; Ryals et al., 1985). (This area is covered in more detail in Section 2.2,

Inducers and Transcriptional Control). The 3′ flanking regions vary in length and contain several polyadenylation sites and thus can give rise to mRNAs of different lengths (Mantei and Weissmann, 1982; Henco et al., 1985). They contain above-average numbers of the sequence motifs ATTA or TTATTTAT. Such sequences are common, however, in many other cytokine genes and other genes, such as protooncogenes, that are inducibly and transiently expressed. It has been proposed that these sequences contribute to the relative instability and short half-lives of IFN and cytokine mRNAs (Caput et al., 1986; Shaw and Kamen, 1986).

2.2   INDUCERS AND TRANSCRIPTIONAL CONTROL

All IFN genes are normally silent and thus require some sort of stimulus to induce expression. A wide range of

-220
AAAACAAAACATTTGAGAAACACGGCTCTAAACTCATGTAAAGAGTGCATGAAGGAAAGCAAAA
                                                          ******
ACAGAAATGGAAAGTGGCCCAGAAGCATTAAGAAAGTGGAAATCAGTATGTTCCCTATTTAAGG

CATTTGCAGGAAGCAAGGCCTTCAGAGAACCTAGAGCCCAAGGTTCAGAGTCACCCATCTCAGC
                                    +1
AAGCCCAGAAGTATCTGCAATATCTACGATGGCCTCGCCCTTTGCTTTACTGATGGTCCTGGTG

GTGCTCAGCTGCAAGTCAAGCTGCTCTCTGGGCTGTGATCTCCCTGAGACCCACAGCCTGGATA

ACAGGAGGACCTTGATGCTCCTGGCACAAATGAGCAGAATCTCTCCTTCCTGTCTGATGGACAG

ACATGACTTTGGATTTCCCCAGGAGGAGTTTGATGGCAACCAGTTCCAGAAGGCTCCAGCCATC

TCTGTCCTCCATGAGCTGATCCAGATCTTCAACCTCTTTACCACAAAAGATTCATCTGCTGCTT

GGGATGAGGACCTCCTAGACAAATTCTGCACCGAACTCTACCAGCAGCTGAATGACTTGGAAGC

CTGTGTGATGCAGGAGGAGAGGGTGGGAGAAACTCCCCTGATGAATGCGGACTCCATCTTGGCT

GTGAAGAAATACTTCCGAAGAATCACTCTCTATCTGACAGAGAAGAAATACAGCCCTTGTGCCT

GGGAGGTTGTCAGAGCAGAAATCATGAGATCCCTCTCTTTATCAACAAACTTGCAAGAAAGATT
   560
AAGGAGGAAGGAATAACATCTGGTCCAACATGAAAACAATTCTTATTGACTCATACACCAGGTC

ACGCTTTCATGAATTCTGTCATTTCAAAGACTCTCACCCCTGCTATAACTATGACCATGCTGAT
                700
AAACTGATTTATCTATTTAAATATTTATTTAACTATTCATAAGATTTAAATTATTTTTGTTCAT
                                              800
ATAACGTCATGTGCACCTTTACACTGTGGTTAGTGTAATAAAACATGTTCCTTATATTTATATT

(a)   TACTCAAAAAAAA

α1 AGAGTGCATGAAGGAAAGCAAAAAGAGAAATGGAAAGTGGCCCAGAAGCATT

AAGAAAGTGGAAATCAGTATGTTCCCTATTTAA

NFκB

ATF/CREB   IRF-1/IRF-2   PRD II
       PRD IV   PRD III   PRD I

     -100
β   AAAATGTAAATGACAGAGGAAAATTGAAAGGGAGAAGTGAAAGTGGGAAATT

CCTCTGAATAGAGAGAGGACCATATAGTATAAA

ω   GGGAGAACACACAAATGAAAACAGTAAAAGAAACTGAAAGTACAGAGAAATG

TTCAGAAAATGAAAACCATGTGTTTCCTATTAA

(b)

Figure 25.1 (a) Nucleotide sequence of the chromosomal IFN-α₁ coding segment and its flanking regions. The coding sequence including the signal sequence is highlighted. The TATA box in the 5′ flanking region is marked by asterisks. (b) A comparison of the promoter regions for IFN-α₁, IFN-β, and IFN-ω genes. The sequences upstream of the TATA box (underlined) of the IFN-α₁, IFN-β, and IFN-ω promoters are aligned. The positions of GAAANN hexamers are indicated by hatched boxes. The regions of the IFN-β promoter thought to be required for binding of transcription factors are indicated by named shaded boxes.

inducers, including viruses, bacteria, mycoplasma, endotoxins, double-stranded polynucleotides or RNA (dsRNA), and some cytokines, have been shown to efficiently activate transcription of IFN genes (Stewart, 1979; De Maeyer and De Maeyer-Guignard, 1988). Such inducers have in general the potential to induce

expression of all IFNA, IFNB, and IFNW genes; however, there appears to be cell- and inducer-specific selectivity that governs the type and numbers of IFN genes expressed. For instance, virally induced human diploid fibroblasts produce mainly IFN-β and only a minor amount of IFN-α (Havell et al., 1978), whereas virally induced PBMC produce mainly IFN-α plus IFN-ω and only a minor amount of IFN-β (Cantell and Hirvonen, 1977; Adolf et al., 1990). This has to some extent been confirmed at the mRNA level (Shuttleworth et al., 1983; Hiscott et al., 1984). Differences have also been reported in the proportions of individual IFN-α subtypes produced by different cell types (Goren et al., 1986; Finter, 1991; Greenway et al., 1992), suggesting that IFNA genes may be differentially expressed. However, the way in which such differential expression is regulated is presently not understood.

Transcriptional control of IFNA genes resides in their 5′ flanking region, upstream from the cap site. Nucleotide deletions outside of position −117 from the cap site have little impact on transcriptional control, but deletions farther in eliminated induction, indicating that this region, −117 to −1, contained promoter regulatory elements (Ragg and Weissmann, 1983; Weidle and Weissmann, 1983). These have been further delineated as a purine-rich nucleotide tract between −109 and −64, containing hexameric repeats of GAAANN (GAAA G/C T/C), which appears to be necessary for inducible transcription and which has been termed the "virus-regulating element" (VRE) (Ryals et al., 1985).

Similar studies involving 5′ deletions have been carried out with the IFNB gene, and it has been found that 5′ sequences within −110 to −1 contain regulatory elements that are required for induction by viruses and dsRNA. The minimum VRE has been localized to −74 to −37 with respect to the cap site (Goodbourn et al., 1986; Goodbourn and Maniatis, 1988) and contains two positive virus-inducible elements, termed positive regulatory domains (PRDI, −77 to −64; PRDII, −66 to −55), and a negative regulatory domain (NRD, −57 to −37) (Figure 25.1) (Fujita et al., 1985; Fan and Maniatis, 1989; Whittemore and Maniatis, 1990; Nourbakhsh et al., 1993). In addition, the hexameric repeat sequences GAAANN (also present in IFNA genes) spanning from −110 to −65 contain variants of the PRDI sequence and two further regulatory elements, PRDIII (−90 to −78) and PRDIV (−104 to −91) have been identified that are required for a functional VRE in IFNB gene expression in mouse L cells (Dinter and Hauser, 1987; Du and Maniatis, 1992). PRDI and PRDIII act as binding sites for a nuclear transcription factor, designated "interferon regulatory factor-1" (IRF-1) (Miyamoto et al., 1988), whose expression is transiently increased by virus infection and which appears to mediate the activation of transcription of the IFNB gene (Fujita et al., 1988; Harada et al., 1989; Xanthoudakis et al., 1989). A second virus-inducible factor, designated "interferon regulatory

factor-2" (IRF-2), also binds to PRDI but suppresses, rather than activates, transcription (Harada et al., 1989, 1990). PRDII is a binding site for the nuclear transcription factor NFκB (Clark and Hay, 1989; Fujita et al., 1989a; Hiscott et al., 1989; Lenardo et al., 1989; Visvanathan and Goodbourn, 1989), which interacts with the major groove of the DNA (Thanos and Maniatis, 1992). Additionally, another protein, high-mobility group Y/1, also binds to PRDII, interacting with the minor groove of the DNA (Thanos and Maniatis, 1992). Both factors appear to be necessary for virus induction of the IFNB gene promoter. PRDIV contains a binding site for a protein of the cAMP response element binding protein (ATF/CREB) family of transcription factors (Du and Maniatis, 1992).

Viral induction of the IFNB gene is thought to occur following activation of pre-existing NFκB and by de novo synthesis of IRF-1; these nuclear transcription factors bind to the tandemly arranged PRDI and PRDII and act cooperatively to initiate/activate transcription (Leblanc et al., 1990; Lenardo et al., 1989; Visvanathan and Goodbourn, 1989; Fujita et al., 1989b; Watanabe et al., 1991). Reporter constructs containing PRDI supported by a simian virus 40 (SV40) enhancer, or $(GAAAGT)_4$, which contains the functional equivalent of dimeric PRDI (Näf et al., 1991) are activated not only by virus but also by overexpression of IRF-1 (Näf et al., 1991; MacDonald et al., 1990). However, in most cell lines, overexpression of IRF-1 has led to poor induction of IFNB (and IFNA) genes (Harada et al., 1990; Fujita et al., 1989b) or none at all (MacDonald et al., 1990; Reis et al., 1992). This has been attributed to the repressive effect of IRF-2, a homologue of IRF-1, which also binds to PRDI (Harada et al., 1989). In the undifferentiated murine embryonal carcinoma (stem) cell line P19, which is refractory to viral induction of IFNB (and IFNA) genes and which expresses neither IRF-1 nor IRF-2, overexpression of an introduced IRF-1 construct leads to activation of endogenous IFN genes and to activation of reporter plasmids with the IFNB promoter (Harada et al., 1990). In addition, cell lines permanently transformed with an antisense IRF-1 expression plasmid exhibited strongly reduced IFNB gene inducibility that nevertheless could be restored by transient transformation with an IRF-1-overproducing expression plasmid (Reis et al., 1992). However, the role of IRF-1 in virus-induced activation of the IFNB promoter remains controversial (Whiteside et al., 1992; Pine et al., 1990) and Ruffner and colleagues (1993) have shown that in murine embryonal stem cells devoid of both IRF-1 gene alleles (IRF-1 $^0/_0$) viral induction of IFNB was only slightly higher in control IRF-1 $^0/_+$ differentiated stem cells than that in IRF-1 $^0/_0$ differentiated cells. This suggests that while IRF-1 at high levels may elicit or enhance induction of IFNB under certain circumstances, it is not essential for viral induction. In cultured mouse fibroblasts devoid of IRF-1, IFNB induction by the

synthetic dsRNA molecule poly(I):poly(C) was absent, whereas induction by Newcastle disease virus (NDV) was normal (Matsuyama et al., 1993). However, IFN induction in vivo by either virus or dsRNA has been found to be unimpaired in IRF-1 $^0/_0$ mice, indicating that IRF-1 is not essential (Reis et al., 1994). It has also become clear recently that the PRDI site can bind factors other than IRF-1 and IRF-2, and these may be more important for regulating IFNB gene activation (Whiteside et al., 1992; Keller and Maniatis, 1991).

In contrast, targeted disruption of the IRF-2 gene to yield mouse fibroblasts deficient in the repressor IRF-2 has been found to lead to upregulated induction of IFNB following NDV infection (Matsuyama et al., 1993). This suggests that IRF-2 negatively regulates or represses IFNB gene induction.

The induction of the IFNA1 gene appears to be regulated differently from that of the IFNB gene. IRF-1 is bound poorly by the equivalent PRDI site in the IFNA1 promoter and this promoter also lacks an NFκB site (Figure 25.1) (Miyamoto et al., 1988; MacDonald et al., 1990). The IFNA1 gene VRE does contain a hexameric repeat nucleotide sequence $(GAAATG)_4$, designated a "TG-sequence" (MacDonald et al., 1990), which appears to mediate virus inducibility when supported by an SV40 enhancer, but which does not respond to IRF-1 (Näf et al., 1991). It has, however, been reported that overexpression of IRF-1 can induce IFNA genes, at least under special circumstances (Harada et al., 1990; Au et al., 1992).

The IFNW gene, like IFNA and IFNB genes, is virus inducible and has structural features in its 5′ promoter region similar to those in IFNA/B promoters (Figure 25.1). In particular, hexameric repeat units are present, but are organized differently from those present in IFNA/B genes (Hansen et al., 1991; Roberts et al., 1992). However, the regulation of transcription of the IFNW gene has not been studied in detail.

3. IFN Proteins

3.1 STRUCTURAL FEATURES

3.1.1 IFN-α Subtypes

The 14 IFN-α subtypes are secreted proteins and as such are transcribed from mRNAs as precursor proteins, pre-IFN-α, containing N-terminal signal polypeptides of 23 mainly hydrophobic amino acids (Figure 25.2). The signal polypeptide is cleaved off before "mature" IFN-α molecules are secreted from the cell. From their cDNA sequences, mature IFN-α subtypes have been predicted to contain 166 amino acids (except IFN-α_2, 165 amino acids) (Mantei et al., 1980; Nagata et al., 1980; Streuli et al., 1980; Goeddel et al., 1981; Lawn et al., 1981a,b; Gren et al., 1984). The calculated molecular mass of

recombinant IFN-α subtypes is approximately 18.5 kDa, although apparent molecular masses of leukocyte-derived IFN-α subtypes in sodium dodecyl sulphate (SDS)–polyacrylamide gels vary between 17 and 26 kDa, possibly owing to variable processing of C-terminal amino acids (Levy et al., 1981) and post-translational modifications. The amino acid sequences of IFN-α subtypes are highly related, with complete identity at 85 of the 166 amino acid positions (Langer and Pestka, 1985; De Maeyer and De Maeyer-Guignard, 1988). This is illustrated in Figure 25.2, where the amino acid sequences of the subtypes are compared to an idealized consensus sequence. Many of the positions where amino acids differ from subtype to subtype are conservative substitutions. Interestingly, IFN-α subtypes contain four cysteine residues whose positions (1, 29, 99, and 139) are highly conserved (Figure 25.2). These four cysteines form disulfide bridges (1–99, 29–139) which induce folding of the IFN-α molecule (Figure 25.3) and whose integrity is essential for biological activity (Morehead et al., 1984). IFN-α subtypes are predicted to contain a high proportion (~60%) of α-helical regions and are folded to form globular proteins (Zoon and Wetzel, 1984). It has not yet proved possible to apply x-ray crystallographic techniques to IFN-α subtypes, or to human IFN-β, but the three-dimensional crystal structure of recombinant mouse IFN-β, which is approximately 60% related in amino acid sequences to its human counterpart, has been solved (Senda et al., 1992). This has revealed that mouse IFN-β has a structure which consists of five α-helices folded into a compact α-helical bundle (Figure 25.4). From comparative sequence analysis it is predicted that in all mammalian IFN-α and IFN-β proteins these five α-helical domains are conserved (Korn et al., 1994; Horisberger and Di Marco, 1995) and thus show similarities with many other cytokines, which also have α-helical bundle structures (Bazan, 1990).

With one exception, IFN-α subtypes do not contain recognition sites (Asn-X-Ser/Thr) for N-linked glycosylation (Henco et al., 1985); only IFN-α_{14} contains two of these sites (Figure 25.2). Nevertheless, O-linked glycosylation may be possible in other IFN-α subtypes. For example, it has been found that natural IFN-α_2, purified from human leukocyte IFN, contains the disaccharide galactosyl-N-acetylgalactosamine in O-linkage to Thr-106 (Adolf et al., 1991a). However, since IFN-α_2 is the only IFN-α subtype with a theonine at position 106, it may represent the only O-glycosylated IFN-α protein (Figure 25.2). (N.B. Recombinant IFN-α subtypes produced by E. coli are nonglycosylated since bacteria lack the biosynthetic machinery to add sugar residues to polypeptides; all recombinant IFN-α_2 products used clinically are nonglycosylated.)

3.1.2 IFN-β

Pre-IFN-β contains 187 amino acids, of which 21 comprise the N-terminal signal polypeptide and 166

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                   S1      S10              S20  S23  1         10          20             30          40         50
IFN-α Consensus  MALSFSLLMA VLVLSYKSIC SLG CDLPQTHSLG NRRALILLAQ MGRISPFSCL KDRHDFGFPQ EEFDGNQFQK
IFN -α1          ..SP.A...V LV...C..S. ...  ....E....D ...T.M.... .S....S... M......... ..........
IFN -α2          ...T.A..V. L....C..S. .V.  .......... S..T.M.... .R...L.... .......... ...-......
IFN -α4          .......... .......... ...  .......... .......... .R........ ......E... ....DK....
IFN -α5          ...P.V.... LV...NC.... ...  .........S ...T.MIM.. .......... .......... ..........
IFN -α6          ...P.A.... LV...C..S. ..D  .......... H..TMM.... .R...L.... ......R... ..........
IFN -α7          ..R......V .......... ...  .........R .......... .......... ....E.R..E .....H....
IFN -α8          ...T.Y..V. LV......FS ...  .......... .......... .R........ ......E... ....DK....
IFN -α10         .......... .........* ...  .......T.R .........G. .......... ......RI.. ..........
IFN -α13         ..SP.A...V LV...C..S. ...  ....E....D ...T.M.... .S....S... M......... ..........
IFN -α14         ...P...M.. LV...C..S. ...  .N.S.....N ...T.M.M.. .R........ ......E... ..........
IFN -α16         .......... .......... ...  .......... .......... ....H..... ...Y...... .V........
IFN -α17         .......... .......... ...  .......... .......... .......... ...P...L.. ..........
IFN -α21         .......... .......... ...  .......... .......... .......... .......... ..........
IFN -α22         ...P...M.. LV...C..S. ...  .N.S.....N ...T.MI... .......... .......... ..........
IFN -ω           .V.LLP..V. LPLCHCGPCG ..S  .....N.G.L S.NT.V..H. .R.....L.. ...R..R... .MVK.S.L..
IFN -β           .TNKCL.QI. L.LCFSTTAL .MS  YN.LGFLQRS SNFQCQK.LW QLNGRLEY.. ...MN.DI.E .IKQLQ....


                            60          70          80          90         100         110
IFN -α Consensus  AQAISVLHEM IQQTFNLFST KDSSAAWDES LLEKFYTELY QQLNDLEACV IQEVGVEETP
IFN -α1           .P.......L ...I...T. .........D ..D..C.... .......... M..ER.G...
IFN -α2           .ET.P..... ...I...... .........T ..D..Y.... .......... ...G..T...
IFN -α4           .......... .......... ......L..T .......... .......... ..........
IFN -α5           .......... .......... .....L...T ..D..Y.... .........M M......D..
IFN -α6           .E.......V .......... ....V....R ..D.LY.... .......... M...W.GG..
IFN -α7           T......... .......... E......EQ. .......... .......... ..........
IFN -α8           .......... .......... ......L..T ..DE.YI..D .......VLC D.....I.S.
IFN -α10          .......... .......... E......EQ. ........I. .......... I......II.
IFN -α13          .P.......L ...I...T. .........D ..D..C...C .......... M..ER.G...
IFN -α14          .......... M......... .N.......T .....YI..F ..M....... ..........
IFN -α16          .....AF... .......... .........T ..D..YI..F .......... T.......IA
IFN -α17          T......... .......... E......EQ. .......... ....N..... .....M....
IFN -α21          .......... .......... .....T.EQ. .........N ...M...... ..........
IFN -α22          .......... .......... ......T...T ..D..Y.... .........M M......D..
IFN -ω            .HVM...... L..I.S..H. ER.....NMT ..DQLH...H ...QH..T.L L.V..EG.SA
IFN -β            ED.ALTIY.. L.NI.AI.RQ DS..TG.N.T IV.NLLANV. H.I.H.KTVL EEKLEK.DFT


                           120         130         140         150         160         166
IFN- α Consensus  LMNEDSILAV RKYFQRITLY LTEKKYSPCA WEVVRAEIMR SFSFSTNLQK RLRRKD------
IFN -α1           ...A...... K...R..... .......... .......... .L.L.....E .....E
IFN -α2           ..K....... .......... .K........ .......... ...L.....E S..S.E
IFN -α4           .......... .......... .I.R...... .......... .L........ ......
IFN -α5           ...V....T. .......... .......... .......... ...L.A...E .....E
IFN -α6           .......... .......... .......... .......... ...S.R...E ....E
IFN -α7           .....F.... .......... .M........ .......... ........K. G.....
IFN -α8           ..Y....... .......... .......S.. .......... ...L.I.... ..KS.E
IFN -α10          .......... .......... .I.R...... .......... .L........ ......
IFN -α13          ...A.....L K...R..... .......... .......... .L.L.....E .....E
IFN -α14          .......... K......... .M........ .......... .......... G.....
IFN -α16          .......... .......... .MG....... .......... .......... G.....
IFN -α17          .......... .......... .......... .......... .L........ I.....
IFN -α21          ...V...... K......... .......... .......... ..L.KIF.E .....E
IFN -α22          ...V...T. .......... .......... .......... ...L.A...E .....E
IFN -ω            GAISSPA.TL .R...G.AV. .K.....D.. .....M...K .LFL...M.E ...S.DADLGSS
IFN -β            RGKLM.S.HL KR.YG..LH. .KA.E..H.. .TI..V..L. N.Y.INR.TG Y..N
```

Figure 25.2   Amino acid sequence comparison of human IFN-α subtypes, IFN-β, and IFN-ω. All sequences are shown in comparison to an IFN-α consensus subtype sequence obtained by computer analysis of IFN-α subtype sequences. Signal sequences are marked S. Sites of N-linked glycosylation are underlined.

comprise the mature IFN-β protein (Derynck et al., 1980, 1981; Taniguchi et al., 1980; Houghton et al., 1981). Although IFN-β is the same length as the majority of IFN-α subtypes, it shows only approximately 30% amino acid sequence relatedness with them (Figure 25.2) and is antigenically distinct. The IFN-β protein lacks the N-terminal Cys-1 residue present in IFN-α subtypes, but contains three other cysteines at positions 17, 31, and 141, the latter two corresponding to the

disulfide bond pairing 29–139 in IFN-α subtypes. Replacement of Cys-17 by serine does not result in any loss of biological activity, whereas serine substitution of Cys-141 does (Mark et al., 1981; Shepard et al., 1981). As mentioned previously, on the basis of the three-dimensional structure of recombinant mouse IFN-β (Figure 25.4), human IFN-β is predicted to contain five α-helices and to fold up into an α-helical bundle structure (Senda et al., 1992).
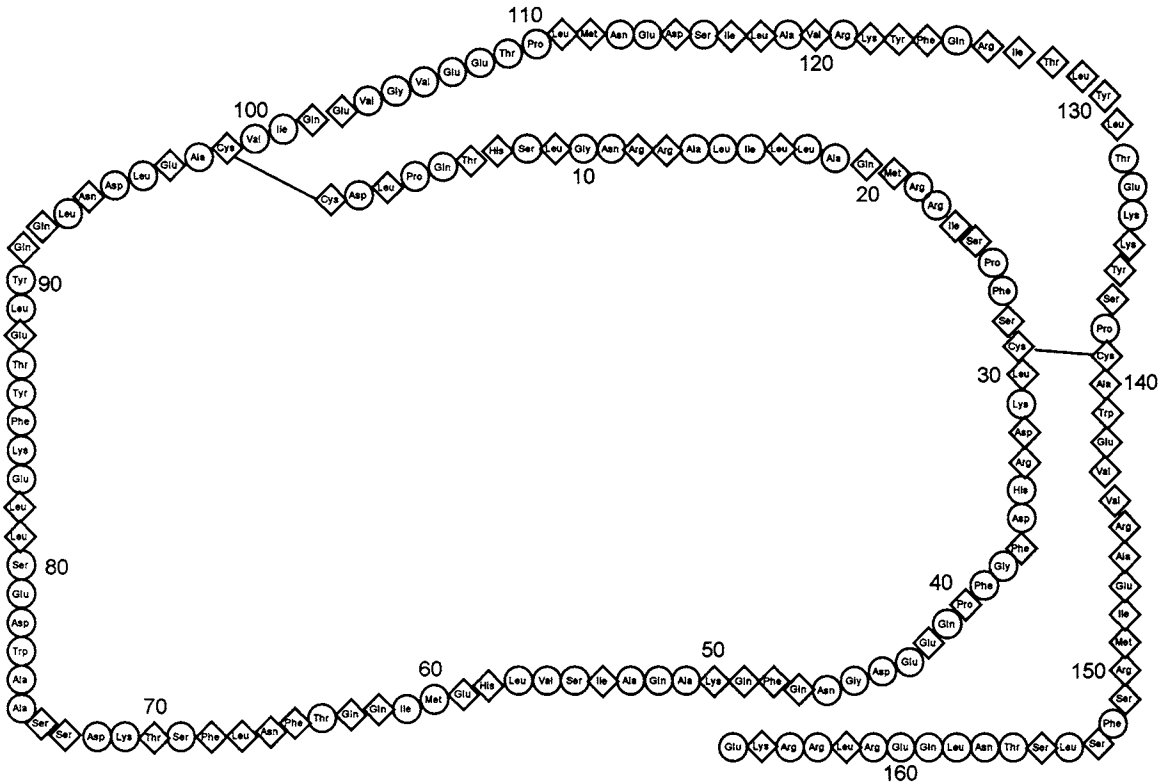
Figure 25.3   Consensus amino acid sequence of human IFN-α. Residues in squares are common to all known IFN-α subtypes.

Human IFN-β has one potential N-glycosylation site at Asn-80 (Taniguchi *et al.*, 1980) and N-linked oligosaccharides, primarily of the biantennary complex-type, are known to be attached to this site in natural IFN-β (Hosoi *et al.*, 1988). However, these may vary considerably depending on the producer cell type (Utsumi *et al.*, 1989).

3.1.3   IFN-ω

From cDNA sequence data, it was predicted that pre-IFN-ω contains 195 amino acids, the N-terminal 23 comprising the signal sequence and the remaining 172 the mature IFN-ω protein (Capon *et al.*, 1985; Hauptmann and Swetly, 1985). The amino acid sequence of IFN-ω is therefore six residues longer at the C-terminus than IFN-α or IFN-β proteins. However, it has been found that natural IFN-ω is heterogeneous at the N-terminus owing to variable cleavage of pre-IFN-ω; about 60% of mature IFN-ω molecules carry two additional N-terminal amino acids (Adolf, 1990; Shirono *et al.*, 1990). It is approximately 60% related to IFN-α subtype sequences, but only 30% related to that of IFN-β (Figure 25.2), and is antigenically distinct from both IFN-α and IFN-β (Adolf, 1990). Nevertheless, the four cysteines occur in the same notional positions, 1, 29,

99, and 139, as they do in IFN-α subtypes and it is likely that IFN-ω will have a similar α-helical bundle structure to those predicted for both IFN-α and IFN-β proteins (Senda *et al.*, 1992). IFN-ω has one potential site at Asn-78 for N-linked glycosylation and natural IFN-ω has been demonstrated to be a glycoprotein with biantennary complex oligosaccharides (containing neuraminic acid) attached at this site (Adolf, 1990; Adolf *et al.*, 1991b).

4.   Cellular Sources and Production

Type I IFNs (IFN-α, -β, and -ω) are produced by a variety of normal cell types responding to extracellular or intracellular stimuli (Stewart, 1979). IFN-α, as a mixture of subtypes, and IFN-ω may be produced together following viral infection of null lymphocytes or monocytes/macrophages (Cantell and Hirvonen, 1977; Adolf, 1990). The proportions of IFN-α subtypes may vary according to the type of virus used as inducer (Hiscott, 1984; Finter, 1991). However, production of IFN-β is usually restricted to double-stranded polynucleotide, e.g., poly-inosinic, poly-cytidylic acid, or
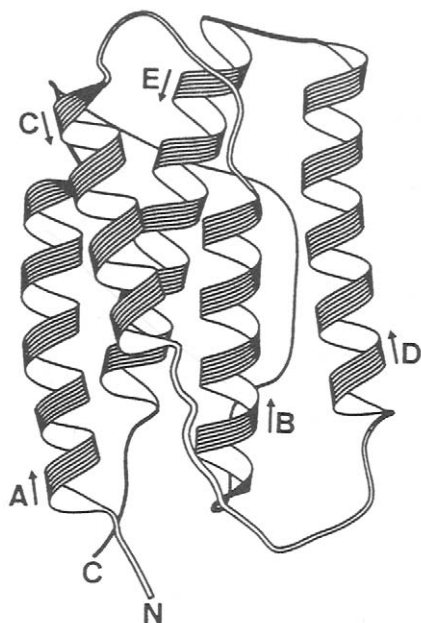
Figure 25.4   Ribbon drawing of the Cα structure of mIFN-β (Senda et al., 1992). The view is perpendicular to the helical axes. Helices A, B, C, D, and E, as well as NH₂- and COOH-termini, are labeled. The arrows indicate the direction of the helices. (Reprinted with permission of Horisberger and Di Marco (1995) Pharmac. Ther. 66, 535. Copyright Elsevier Science Ltd., Amsterdam, The Netherlands.)

virally-induced normal fibroblasts and other tissue cell types, e.g., epithelial cells (Meager et al., 1979; Stewart, 1979). In all the above cases, the amount of IFN secreted is dependent on the dose of the inducer.

Besides normal cells, a range of transformed and tumor-derived cell lines are known IFN producers, e.g., MG63 human osteosarcoma cell line (Meager et al., 1982), and the Namalwa B-lymphoblastoid cell line (Phillips et al., 1986). Generally speaking, adherent fibroblastic cell lines produce mainly IFN-β and only a minor quantity of IFN-α (Havell et al., 1978), whereas nonadherent myeloid or lymphoid cell lines produce mainly IFN-α and only a small amount of IFN-β (Cantell and Hirvonen, 1977; Shuttleworth et al., 1983; Zoon et al., 1992).

Actual production of IFNs lasts only a matter of a few hours following induction. This is due to IFN mRNA instability and the rapid shut-off of IFN gene transcription (Caput et al., 1986; Shaw and Kamen, 1986). Under conditions where IFN mRNA stability is increased, e.g., by blocking protein and RNA synthesis following induction, IFN production has been shown to be "superinduced" once the block on protein synthesis is removed (Meager et al., 1979; Stewart, 1979).

5. Biological Activities Associated with IFN-α/β/ω

It is well established that the biological activities of IFNs are mostly dependent upon protein synthesis with selective subsets of proteins mediating individual activities. Antiviral, antiproliferative and immuno-modulatory activities have been ascribed to IFN-α/β/ω (reviewed in Pestka et al., 1987; De Maeyer and De Maeyer-Guignard, 1988). The proteins and mechanisms involved in these activities are described below.

5.1   ANTIVIRAL ACTIVITY

5.1.1   Molecular Mechanisms

Despite there being vast numbers of viruses with different replication strategies, it appears that many viruses can be countered by relatively few IFN-inducible "antiviral" proteins (Samuel, 1987). One of the best-characterized of these is a family of enzymes collectively known as "2-5A synthetase" which, in the presence of dsRNA (often an intermediate of viral RNA synthesis) catalyses the formation of an unusual oligonucleotide, ppp (A2′p) nA (2-5A), which in turn activates an IFN-induced latent endonuclease, RNase L (Williams and Kerr, 1978; Wreschner et al., 1981; Ghosh et al., 1991; Hovanessian, 1991; Lengyel, 1982; Zhou et al., 1993). When activated, the RNase L degrades viral (and cellular mRNA) and therefore inhibits viral protein synthesis. Small RNA viruses (picornaviridae), e.g., Mengo virus and murine encephalomyocarditis virus (EMCV), whose replication is cytoplasmic are most inhibited by the induction of 2-5A synthetase (Lengyel, 1982; Rice et al., 1985; Chebath et al., 1987; Kumar et al., 1988). A further important IFN-induced "antiviral" protein is a dsRNA-dependent protein kinase, now designated PKR, which in the active form phosphorylates the peptide initiation factor, eIF2, involved in polyribosomal translation of mRNA (Miyamoto and Samuel, 1980; Gupta et al., 1982; Samuel, 1987). Phosphorylated eIF2 is inactive and thus viral protein synthesis is inhibited. This inhibition has been associated with the loss of replicating capacity of reoviruses and rhabdoviruses such as vesicular stomatitis virus (VSV).

The 2-5A synthetase and PKR antiviral mechanisms are rather general and potentially could affect a wide range of viruses. However, IFN can induce certain proteins that inhibit specifically one class of virus. For example, the IFN-inducible Mx proteins block the replication of influenza virus, probably by inhibiting the nuclear phase of viral transcription (mouse cells) or later cytoplasmic phases (human cells), without affecting the replication of many other viruses (Staeheli, 1990; Melén et al., 1992; Ronni et al., 1993).

In addition, since IFNs can impair various steps of viral replication, including penetration, uncoating and

assembly of progeny virions as well as transcription and translation, there are likely to be several other antiviral proteins and mechanisms (De Maeyer and De Maeyer-Guignard, 1988). For instance, some viruses, e.g., herpes virus and certain retroviruses, appear to be inhibited at the relatively late stage of virus particle maturation and budding (Aboud and Hassan, 1983).

5.1.2 Defense Mechanisms of Viruses

In the course of evolution, many viruses have developed countermechanisms by which they can disrupt the antiviral mechanisms induced by IFN. Such countermechanisms often point to the significance of particular "antiviral" proteins. One of the main "targets" for several different viruses is the IFN-inducible PKR. The action of this kinase is overcome in adenovirus or Epstein–Barr virus (a member of the herpes virus family) by the production of small viral RNA molecules, VAI- and EBER-RNAs, respectively, which bind to PKR and block its activation by dsRNA (Clarke et al., 1991; Ghadge et al., 1991; Mathews and Shenk, 1991). Reoviruses and vaccinia virus (a member of the pox virus family) produce viral proteins (sigma3 and SKI, respectively), that bind to dsRNA and thus reduce activation of PKR (Sen and Lengyel, 1992). Interestingly, if IFN-treated VSV-infected cells are co-infected by vaccinia virus, VSV replication is rescued, presumably partly by the inhibitory effect of SKI on PKR (Whitaker-Dowling and Youngner, 1983). Vaccinia virus also produces a nonfunctional protein analog of eIF2 which competes with the real eIF2 for phosphorylation by PKR and thus dilutes out the antiviral effect of activated PKR (Beattie et al., 1991). Other viruses, such as influenza, may activate latent cellular inhibitors of PKR activity, e.g., a 58 kDa protein (p58) (Lee et al., 1992).

The 2-5A synthetase–RNase L system can also be subverted. For example, EMCV, a picornavirus, can inactivate RNase L in several cell lines, but this inactivation is usually blocked by IFN treatment (Lengyel, 1982). Herpes viruses, in contrast, appear to inhibit RNase L activation by producing competing analogs of 2-5A (Cayley et al., 1984).

Some viruses even have the ability to block the transcription of IFN-inducible genes. The "early" E1a regulatory proteins of adenoviruses prevent the activation of ISGF3 by IFN, probably by inhibiting the transcription of the ISGF3γ subunit (Ackrill et al., 1991; Gutch and Reich, 1991; Kalvakolanu et al., 1991; Nevins, 1991). In the case of hepatitis B virus-infected cells, the so-called virus-specified "terminal protein" inhibits IFN-inducible gene expression (Foster et al., 1991).

5.2 ANTIPROLIFERATIVE ACTIVITY

The antiviral mechanisms induced by IFN are mediated by enzymes, e.g., 2-5A synthetase and PKR, whose activities have broad implications for cell growth and proliferation. Viral replication may be regarded as a form of pathological growth of a foreign, "cell-like", entity at the expense of a living cell. In the presence of IFN, enzymes are activated which curtail protein synthesis in general, but because viral protein synthesis is normally rapid, the inhibitory effect on viral replication appears more dramatic than on the slower and more complex cellular growth. Possibly, the "IFN system" was evolved more as a part of a complex, interactive network of intercellular mediators of cell growth and proliferation than as one for antiviral mechanisms. Recent investigations tend to support the role of IFNs in regulating cell growth. For example, if a mutant form of PKR that is unable to phosphorylate eIF2 is introduced into cells, they undergo neoplastic transformation (Koromilas et al., 1992; Lengyel, 1993; Meurs et al., 1993). This suggests that PKR normally acts as a "tumor-suppressor"' gene product. Therefore, one of the mechanisms by which IFN inhibits cell proliferation could be through its capacity to induce enhanced expression/activity of PKR. IFN-α has also been reported to inhibit cyclin-dependent CDK-2 kinase, which is responsible for phosphorylation of retinoblastoma (RB) protein, and this could contribute to antiproliferative activity (Kumar and Atlas, 1992; Resnitzky et al., 1992; Zhang and Kumar, 1994).

The 2-5A synthetase–RNase L system may also have antiproliferative and tumor suppressor activities. For instance, the levels of these two enzymes are high in growth-arrested cells: introduction of 2-5A-like oligoadenylates into proliferating cells also causes growth impairment (Sen and Lengyel, 1992; Lengyel, 1993; Zhou et al., 1993).

The IFN-stimulated increases in synthesis of PKR and 2-5A synthetase are dependent on IFN-inducible transcription factors, such as IRF-1 (ISGF2) (Miyamoto et al., 1988; Pine et al., 1990; Williams, 1991; Reis et al., 1992). The latter has a short half-life and thus transcription of IFN-inducible genes is rapidly repressed by the longer-lasting, inhibitory IRF-2 (Harada et al., 1989). If IRF-2 is overexpressed, cells become transformed as even low level constitutive production of PKR and 2-5A synthetase, which can regulate normal cell growth, is abrogated. This transformation was reversed by overexpressing IRF-1 (Harada et al., 1993), indicating that IRF-1 can be viewed as a pivotal player in the growth, regulatory, and tumor suppressor machinery. A variety of other IFN-induced mechanisms, including suppression of oncogenes (Contente et al., 1990), depletion of essential metabolites (Sekar et al., 1983), and increased cell rigidity (E. Wang et al., 1981), could also contribute to its antiproliferative activity.

The antiproliferative effects of IFNs in different tumor cell lines cultured in vitro is highly variable. Besides tumor cell lines, IFN-α/β have antiproliferative activity in hematopoietic precursor cells, e.g., of the myeloid

lineage (Rigby *et al.*, 1985; De Maeyer and De Maeyer-Guignard, 1988, for review). They are also potent inhibitors of angiogenesis, the process whereby blood capillaries are formed to envasculate tissues (Sidky and Borden, 1987).

5.3 IMMUNOREGULATORY ACTIVITY

Besides activating intracellular processes, IFNs can also activate intercellular activities, especially within the immune system, which are an essential part of host defense against infectious and invasive diseases. Thus, IFNs can stimulate indirect antiviral and antitumor mechanisms, which in the main rest upon cellular differentiation and the induction of cytotoxic activity. For example, in the presence of antigen-specific antibodies, macrophages can effect cell-mediated cytotoxicity. Such antibody-dependent cell-mediated cytotoxicity (ADCC) is enhanced by IFN, possibly through an augmentation of immunoglobulin G (IgG)-Fc receptor (FcR) expression (Hokland and Berg, 1981; Vogel *et al.*, 1983; De Maeyer and De Maeyer-Guignard, 1988). In addition, another category of leukocytes comprising large granular lymphocytes and known as natural killer (NK) cells are activated, by unknown mechanisms, to kill virally-infected or tumor cell targets independently of major histocompatibility complex (MHC) antigen expression (Trinchieri and Perussia, 1984; Rager-Zisman and Bloom, 1985; De Maeyer and De Maeyer-Guignard, 1988).

IFNs can stimulate increased expression of class I MHC antigens, i.e., HLA-A, -B, -C, which are crucial for recognition of foreign antigen by cytotoxic T lymphocytes (CTL, CD8$^+$); recognition of virally infected cells by CTL depends on class I MHC antigen presentation of viral antigens at the cell membrane (Heron *et al.*, 1978; Fellous *et al.*, 1979). IFN-α/β have sometimes been observed to increase class II MHC antigen expression, which is necessary to trigger both humoral and cell-mediated immunity, but probably play a lesser role than IFN-γ, which is the major class II MHC antigen inducer (Baldini *et al.*, 1986; Rhodes *et al.*, 1986; De Maeyer and De Maeyer-Guignard, 1988).

5.4 BIOLOGICAL ACTIVITIES *IN VIVO*

All of the biological activities so far described (see above) for IFNs have followed from *in vitro* experimentation. Here it is possible to pick and choose conditions that favor particular outcomes, e.g., the antiviral response, by adjusting doses of IFN, times of incubation, levels of virus challenge, and so on. Such experiments illustrate the range of biological activities of IFNs but cannot define their physiological roles. That IFNs have the potential for inducing antiviral and antitumor activity suggests their main role *in vivo* is to act as regulators of

host defense mechanisms, and to prevent pathophysiological events occurring. Investigations in experimental animals have supported this likelihood. For example, injection of mice with anti-IFN-α/β antibody has been shown to increase their susceptibility to a range of virus infections (Virelizier and Gresser, 1978; Gresser, 1984). The earliest evidence for an antitumor effect of IFN-α/β came from inoculation of murine L1210 cells into mice. L1210 cells are sensitive to the antiproliferative action of IFN-α/β *in vitro* and *in vivo*, IFNα/β prevented tumor growth by these cells. However, when a clone of L1210 was isolated that was resistant to the antiproliferative action of IFN-α/β, there occurred a similar retardation of tumor growth upon IFN-α/β treatment to that observed with "sensitive" L1210 cells, suggesting that IFN was acting indirectly *in vivo* by a host-mediated mechanism (Gresser *et al.*, 1970, 1972). A similar conclusion was reached more recently using IFN-resistant B-cell lymphoma cells (Reid *et al.*, 1989). In the intervening years, many studies have been conducted confirming that IFN can act directly (e.g., human IFN-α_2 against a range of human tumor xenografts in nude mice where human IFN-α_2 has no activity on the murine immune system) and indirectly (reviewed by Balkwill, 1989). Although antitumor activity has been clearly demonstrated by the application of exogenous IFNs, it is not certain that endogenously produced IFNs are involved in countering tumor growth. However, some experimental evidence that endogenous IFN could play a role in host resistance to cancer or its spread has been obtained by treating mice with anti-IFN antibodies. Under these conditions, the intraperitoneal transplantability of six different experimental murine tumors was observed (Gresser, 1984).

IFN-α/β can inhibit the growth of hematopoietic progenitor cells *in vitro* (Rigby *et al.*, 1985) and this is also likely to occur *in vivo*. Such an occurrence is undesirable in most instances, but suppression of proliferation of bone marrow hematopoietic cell precursors has been turned to advantage in protecting tumor-bearing mice against the cytotoxicity of chemotherapeutic agents such as 5-fluorouracil (5-FU) (Stolfi *et al.*, 1983).

6. Receptors

6.1 CHARACTERIZATION

6.1.1 General Features

IFNs exercise their actions in cells via IFN-specific cell surface receptors. These receptors bind IFNs with high affinity (Aguet, 1980) and transduce the signal occasioned by ligand (IFN) binding across the cell membrane into the cytoplasm. IFN-α, IFN-β and IFN-

ω share the same binding sites (Aguet *et al.,* 1984; Flores *et al.,* 1991), but IFN-γ binds to different sites (Branca and Baglioni, 1981; Aguet *et al.,* 1988). The binding of IFN-α and IFN-β to lymphoid cells and fibroblasts has been studied extensively and has been reviewed (Rubinstein and Orchansky, 1986; Branca, 1988; Langer and Pestka, 1988; Grossberg *et al.,* 1989), and results have generally demonstrated the presence of up to a few thousand complex, high-affinity receptors per cell. Chemical cross-linking studies with ^{125}I-labeled IFN-α or IFN-β to receptor-bearing cells have led to the identification of various IFN–receptor complexes, their molecular masses ranging from 80 to 300 kDa (Joshi *et al.,* 1982; Eid and Mogensen, 1983; Faltynek *et al.,* 1983; Raziuddin and Gupta, 1985; Thompson *et al.,* 1985; Hannigan *et al.,* 1986; Vanden Broecke and Pfeffer, 1988; Colamonici *et al.,* 1992). Such results have suggested that there are either multiple binding sites for IFN-α, IFN-β, and IFN-ω or that there are complex multichain receptors (Colamonici *et al.,* 1992; Hu *et al.,* 1993).

Although on human cells all the IFN-α, IFN-β, and IFN-ω proteins compete for common binding sites, individual IFN-α subtypes show different levels of activities on cells (Streuli *et al.,* 1981; Weck *et al.,* 1981; Rehberg *et al.,* 1982) which appear to correlate with their binding behavior to the cell surface (Uzé *et al.,* 1985). In particular, IFN-α_1 shows a much lower binding for human membrane receptors than either IFN-α_2 or IFN-α_8 (Uzé *et al.,* 1988). Interestingly, this differential binding of human IFN-α subtypes is not manifested in bovine cells, and all of the subtypes exhibit high specific activities (Yonehara *et al.,* 1983; Shafferman *et al.,* 1987). IFN-β and IFN-ω are also active in bovine cells (Capon *et al.,* 1985; Adolf *et al.,* 1990), but this cross-reactivity does not extend to mouse cells, a feature that has provided experimental systems in which to characterize IFN-receptors. Thus, somatic cell genetic studies with human × rodent hybrid cells containing various combinations of human chromosomes have provided evidence that the presence of human chromosome 21 confers sensitivity of such hybrid "rodent" cells to human IFN-α, IFN-β and IFN-ω (Tan *et al.,* 1973; Slate *et al.,* 1978; Epstein *et al.,* 1982; Raziuddin *et al.,* 1984). Further, it was demonstrated that antibodies raised against human chromosome 21-encoded cell surface proteins were able to block the binding and action of human IFN-α to human cells, indicating that this chromosome contained a gene(s) specifying the human IFN cell surface receptor (Shulman *et al.,* 1984).

6.1.2   Molecular Cloning of IFN Receptor Components

The elucidation of the full complement of components of the IFN-α/β/ω receptor has long been sought. One methodology used to isolate receptor cDNAs involves

transfecting mouse cells with total human DNA and then selecting for cells sensitive to human IFN-α. After several attempts, this approach led successfully to the isolation of a 2.7 kb cDNA from a library constructed from human lymphoblastoid (Daudi) cells, which encoded an IFN-α binding protein (Uzé *et al.,* 1990) containing 557 amino acids (molecular mass 63 485 Da) including a signal sequence of 27 mainly hydrophobic amino acids. This protein has a structure typical of a transmembrane glycoprotein: a large N-terminal extracellular domain, which potentially could be highly glycosylated owing to a preponderance of *N*-linked glycosylation sites, a short hydrophobic transmembrane domain, and an intracellular or cytoplasmic tail (Figure 25.5). Its amino acid sequence shows little homology with any currently available sequences of proteins, including the sequence of the human IFN-γ receptor (Aguet *et al.,* 1988); however, the extracellular domain has been predicted to show structural similarities with the latter receptor and to a lesser extent with the so-called hematopoietin receptor supergroup (Bazan, 1990a,b). The gene coding for this putative IFN-α receptor has been mapped to chromosome 21.q22 (Lutfalla *et al.,* 1992), in confirmation of the earlier rodent × human hybrid cell data (Tan *et al.,* 1973; Slate *et al.,* 1978; Epstein *et al.,* 1982; Raziuddin *et al.,* 1984).

Although the cloned "IFN-α receptor" could be shown to confer sensitivity to human IFN-α_8 in transfected mouse cells (Uzé *et al.,* 1990), such cells were relatively insensitive to human IFN-α_2 and human IFN-β. These findings, together with those from anti-IFN-α receptor antibody blocking studies (Colamonici *et al.,* 1990; Revel *et al.,* 1991; Uzé *et al.,* 1991) and affinity cross-linking studies with IFN-α_2 (Colamonici *et al.,* 1992), have suggested that a second IFN-α receptor exists or another component is required besides the cloned IFN-α_8 binding protein, to complete the receptor complex. This hypothesis is further supported by a study (Soh *et al.,* 1994) in which introduction of a yeast artificial chromosome (YAC) containing a segment of human chromosome 21 into chinese hamster ovary (CHO) cells conferred a greatly increased response to both IFN-α_2 and IFN-α_8, as well as an increased response to IFN-β and IFN-ω, whereas the expression of the IFN-α_8 binding protein alone did not confer sensitivity (Revel *et al.,* 1991). However, these increased responses can be "knocked out" by disruption of the IFN-α_8 binding protein gene in the YAC, and then reconstituted by expression of the cDNA encoding the IFN-α_8 binding protein (Cleary *et al.,* 1994), suggesting that cell surface expression of this protein is required for a fully functional receptor (see also Hertzog *et al.,* 1994; Constantinescu *et al.,* 1994).

A second human IFN-α/β receptor, which is probably the additional component of the receptor complex referred to above, has been cloned (Novick *et al.,* 1994). The 1.5 kb cDNA encodes a 331-amino-acid protein,
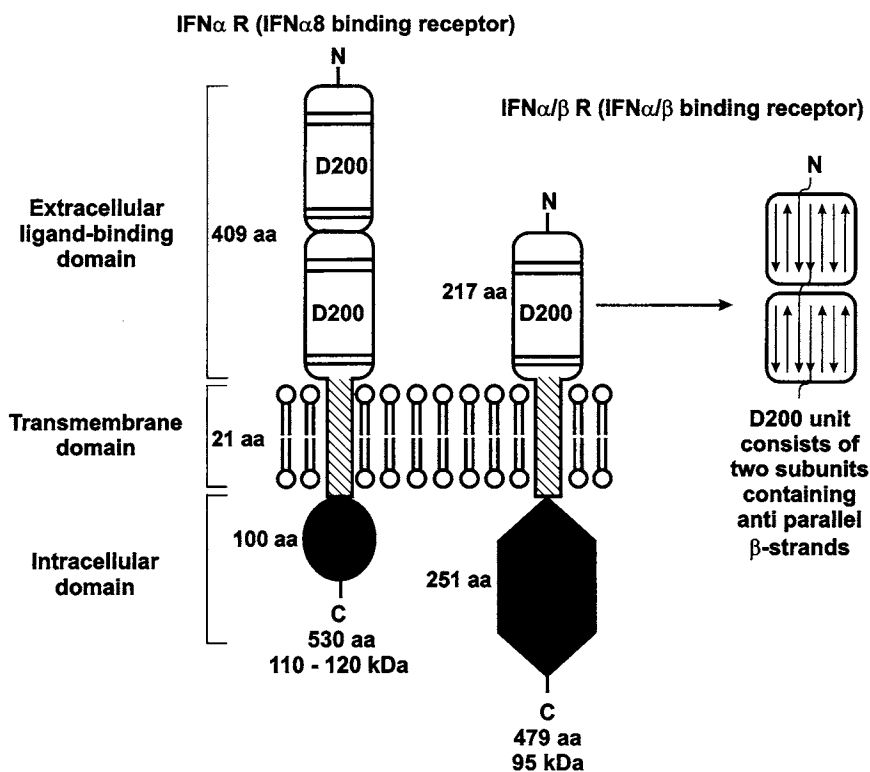
IFNα R (IFNα8 binding receptor)

IFNα/β R (IFNα/β binding receptor)

Figure 25.5   Schematic drawing of the domain organization of the clonal IFN-α_8- and IFN-α/β binding proteins.

including a signal sequence, which has the predicted structure of a transmembrane glycoprotein. The N-terminal ectodomain (217 amino acids) corresponds in sequence to a soluble 40 kDa IFN-α/β binding protein, p40, isolated from urine. This domain is linked to a transmembrane segment (21 amino acids) and a relatively small cytoplasmic domain of 67 amino acids (Figure 25.5). Overall, the primary sequence shows little homology with that of the previously cloned IFN-α_8 binding protein (Uzé et al., 1990), but when the extracellular domains are compared, 23.4% relatedness is found (Novick et al., 1994), suggesting that both of these IFN binding proteins belong to the same so-called class II cytokine receptor family (Uzé et al., 1995). Two classes of cytokine receptor (class I and class II) have been proposed by Bazan (1990a,b), these being distinguished by the positions of cysteine pairs in the extracellular domain. The latter is comprised of fibronectin type III-like units containing around 200 amino acids and designated D200 (Uzé et al., 1995). A schematic drawing of both IFN-α/β receptor chains is shown in Figure 25.5. Subsequently, it has been found that alternative splicing of the IFN-α/β receptor gene can produce a transcript encoding a long form of the receptor protein containing a larger cytoplasmic domain of 251 amino acids (Domanski et al., 1995; Lutfalla et al., 1995).

Mouse cells transfected with the cDNA encoding the

IFN-α/β binding protein bind IFN-α_2 but are insensitive to its effects, suggesting that an accessory protein, possibly the cloned IFN-α_8 binding protein, is required for signaling (Novick et al., 1994; Constantinescu et al., 1994). The findings that anti-p40 antiserum and a particular monoclonal antibody to the IFN-α_8 binding protein (Benoit et al., 1993) both block the biological activity of IFN-αβω indicate that the IFN-α/β and IFN-α_8 binding proteins are in close proximity, and thus probably interact to form a high-affinity IFN-α/β/ω receptor complex. The most likely scenario on present evidence is for a two-chain IFN-α/β/ω receptor, comprising the cloned IFN-α_8 binding protein (Uzé et al., 1990) and the long form of the IFN-α/β binding protein (Domanski et al., 1995; Lutfalla et al., 1995), each of which binds to some extent particular IFN types or IFN-α subtypes but which together more strongly bind all IFN-α, -β, and -ω species and function to transmit signals across the cell membrane. However, it is not completely ruled out that other cell surface components, e.g., membrane glycosphingolipids, are required for fully functional receptors (Colamonici et al., 1992; Platanias et al., 1994; Ghislain et al., 1995; Uzé et al., 1995) or, possibly, that there are alternative IFN receptors, e.g., the Epstein–Barr virus/complement C3d receptor as an IFN-α receptor on B-lymphocytes (Delcayre et al., 1991). Vaccinia virus and other orthopoxviruses contain a gene B18R encoding a soluble

type I IFN receptor which, unlike the class II cytokine type receptors, belongs to the immunoglobulin superfamily (Symons *et al.*, 1995; Colamonici *et al.*, 1995).

7. Signal Transduction

7.1 SIGNAL TRANSDUCTION

7.1.1 Molecular Mechanisms

The intracellular domains of the two cloned IFN-binding proteins are unrelated to the tyrosine kinase class of receptors, e.g., epidermal growth factor receptor (EGF-R) and platelet-derived growth factor-receptor (PDGF-R), and are not predicted to have kinase activity of any sort (Uzé *et al.*, 1990; Novick *et al.*, 1994). However, it appears that the cytoplasmic domain of the IFN-α_8 and α/β binding proteins associate with nonreceptor tyrosine kinases TYK2 and Janus kinase 1 (JAK1), respectively, known to be involved in the signal transduction pathway of IFN-α/β and other cytokines (Novick *et al.*, 1994; Ghislain *et al.*, 1995; Ihle, 1995; Ihle and Kerr, 1995; Velasquez *et al.*, 1995). The current understanding of this pathway is as follows. After binding of IFN-$\alpha/\beta/\omega$ to their cognate receptors, the intracellular domains are phosphorylated by TYK2 and JAK1. These phosphorylated domains act as docking sites for the cytoplasmic STAT (signal transducers and activators of transcription) proteins p84/p91 (STAT1a/b) and p113 (STAT 2) (Ihle, 1996). The latter undergo tyrosine phosphorylation mediated by receptor-associated TYK2/JAK1, dimerize, translocate to the nucleus, and combine with a DNA binding protein, p48, to form the IFN-stimulated gene factor-3 (ISGF3) transcription factor complex (Schindler *et al.*, 1992; Velasquez *et al.*, 1992; Müller *et al.*, 1993; Platanias *et al.*, 1994; Shuai *et al.*, 1994; Gupta *et al.*, 1996; Yan *et al.*, 1996). Both TYK2 and JAK1 need to be reciprocally activated for signal transduction to occur, since cell mutants lacking either TYK2 or JAK1 are unresponsive to IFN-α (Ihle, 1995; Ihle and Kerr, 1995). ISGF3 binds to *cis*-acting IFN-stimulated response elements (ISRE), present in the promoter regions of IFN-inducible genes, to initiate their transcription (Williams, 1991). Targeted disruption of the STAT 1 gene in mice has shown that STAT 1 has an obligatory role in IFN-α and IFN-γ signaling (Durbin *et al.*, 1996; Meraz *et al.*, 1996).

The JAK1/TYK2 – ISGF3 pathway may not be the only "receptor-to-cell nucleus" signaling mechanism activated in IFN-stimulated cells. There has been some evidence to implicate protein kinase C (PKC) pathways as well (Reich and Pfeffer, 1990; Pfeffer *et al.*, 1991; C. Wang *et al.*, 1993). However, this remains controversial owing to the lack of specificity of kinase inhibitors used (Kessler and Levy, 1991; James *et al.*, 1992).

7.2 IFN-INDUCIBLE GENES

7.2.1 IFN-response Gene Sequences

IFN-inducible genes have a common regulatory nucleotide sequence (G/A)GGAAAN(N)GAAACT in their 5′ flanking region and this type of sequence, which resembles the VRE sequences (Ryals *et al.*, 1985; Reid *et al.*, 1989) present in IFN genes, is designated interferon-stimulated response element (ISRE) (Williams, 1991). The resemblance between ISRE and VRE sequences probably accounts for the finding that many IFN-inducible genes are transcriptionally activated by virus infection or dsRNA, which also activate the transcription of IFN genes (Hug *et al.*, 1988; Wathelet *et al.*, 1988). As mentioned previously (see Section 5.1.3), IFN-receptor occupation activates cytoplasmic ISGF-3 and this complex is translocated to the nucleus and binds to ISRE of IFN-inducible genes as a transcriptional activator. In addition, a second factor, ISGF2, forms complexes with ISRE in IFN-stimulated cells. ISGF2 is a single, inducible phosphoprotein that has been shown to be identical to IRF-1 (Miyamoto *et al.*, 1988; Pine *et al.*, 1990; Williams, 1991; Reis *et al.*, 1992). The role of a third transcription factor, ISGF1, which is constitutively produced and requires only the central 9 bp core of ISRE for binding, remains to be fully defined (Kessler *et al.*, 1988). A number of other negative regulatory factors, including IRF2 (Harada *et al.*, 1989) and the ISGF2 (IRF1)/ISGF3γ-related "human interferon consensus sequence binding protein" (ICSBP) (Weisz *et al.*, 1992; Bovolenta *et al.*, 1994), which also bind to ISRE, are also probably involved in the regulation of transcription of IFN-inducible genes.

7.2.2 Proteins Induced by IFN

It is clear that the regulation of expression of IFN-inducible genes is complex and that the mechanisms that control their selective expression are not fully understood (see Taylor and Grossberg, 1990, for review). IFN-inducible proteins, whose number probably exceeds 20, include both those proteins induced early after IFN stimulation and those proteins that may be produced at later times, often in response to the actions of "early" IFN-inducible proteins (Sen and Lengyel, 1992). The full set of IFN-inducible proteins is probably not known, but several have been identified and characterized. Table 25.2 shows an incomplete list of IFN-inducible proteins together with their likely functions. Some of these proteins are not exclusively induced by IFN-$\alpha/\beta/\omega$; IFN-γ and certain other cytokines, e.g., tumor necrosis factor-α (TNF-α) often induce spectra of proteins that overlap with the set induced by IFN-$\alpha/\beta/\omega$ (Revel and Chebath, 1986; Rubin *et al.*, 1988; Wathelet *et al.*, 1992). It should be noted that IFN-inducible proteins tend also to be cell type-specific and thus not all proteins listed in Table 25.2 will be expressed in all cell types. In some cases, IFN-inducible proteins are completely absent from a cell before IFN stimulation, but in other cases

Table 25.2   IFN-inducible proteins

Protein	Function	Reference
2-5A synthetase	dsRNA-dependent synthesis of ppp(A2p)n-A [2-5A]; activator of RNase L	Revel and Chebath, 1986; Sen and Lengyel, 1992; Samuel, 1987; Staeheli, 1990.
dsRNA-activatable protein kinase (PKR)	Phosphorylation of peptide initiation factor eIF-2α	Revel and Chebath, 1986; Sen and Lengyel, 1992.
Class I MHC antigens (HLA-A, B, C) and β-microglobulin	Antigen presentation to cytotoxic T lymphocytes (CTL)	De Maeyer and De Maeyer Guignard, 1988; Heron *et al.*, 1978.
Guanylate-binding proteins (GBP; γ67)	GTP, GDP binding	Schwemmle and Staeheli, 1994.
MxA	Specific inhibition of influenza virus replication	Ronni *et al.*, 1993.
Metallothionein	Metal detoxification	Revel and Chebath, 1986.
Protein kinase C-ε (PKC- ε)	Serine/threonine protein phosphorylation	C. Wang *et al.*, 1993.
Retinoblastoma (RB) gene product	Tumor suppressor protein	Kumar and Atlas, 1992.
15 kDa Ubiquitin cross-reactive protein	Targeting of structurally abnormal proteins for degradation	Loeb and Haas, 1992.
Vimentin	Intermediate filament network	Alldridge *et al.*, 1989.
Tubulin	Cellular structural filaments	Fellous *et al.*, 1982.
IRF1/ISGF2	Nuclear transcription factor	Sen and Lengyel, 1992.
IRF2	Nuclear repressor factor	Sen and Lengyel, 1992.
Interferon-inducible protein 35 (IFP35)	Leucine-zipper type transcription factor	Bange *et al.*, 1994.
Interferon-inducible protein 56 (IFP56)	Unknown	Chebath *et al.*, 1983.
Gene 200 cluster products	204 protein is nucleolar phosphoprotein	Choubey and Lengyel, 1992.
1-8U, 1-8D and 9-27 gene products	9-27 product is an RNA binding protein	Lawn *et al.*, 1981a,b; Constantoulakis *et al.*, 1993.
6-16 gene product	13 kDa hydrophobic protein of unknown function	Porter and Itzhaki, 1993.
Immunoglobulin Fc-receptor (FcR)	Binding of immunoglobulins	Hokland and Berg, 1981.
Intracellular 50 kDa Fcγ-binding protein	Unknown; binds IgG but not IgM, IgA or IgE	Thomas and Linch, 1991.
Thymosin B4	Induction of terminal transferase in B lymphocytes	Revel and Chebath, 1986.

they are being constitutively produced, their synthesis augmented by IFN.

8.   *Mouse IFN-α and IFN-β*

The genes for mouse IFN-α subtypes and mouse IFN-β (no functional mouse IFN-ω gene has been found) are located on mouse chromosome 4 (Dandoy *et al.*, 1984,

1985; De Maeyer and De Maeyer-Guignard, 1988, for review). These genes are, like their human counterparts, intronless and of comparable structure. Twelve mouse IFN-α genes or pseudogenes have been identified, of which the cDNAs for 10 different genes have been cloned and expressed (Langer and Pestka, 1985; De Maeyer and De Maeyer-Guignard, 1988). Mouse IFN-α subtype proteins contain 166 or 167 amino acids, or exceptionally 162 (mouse IFN-α8), and the four cysteines at positions
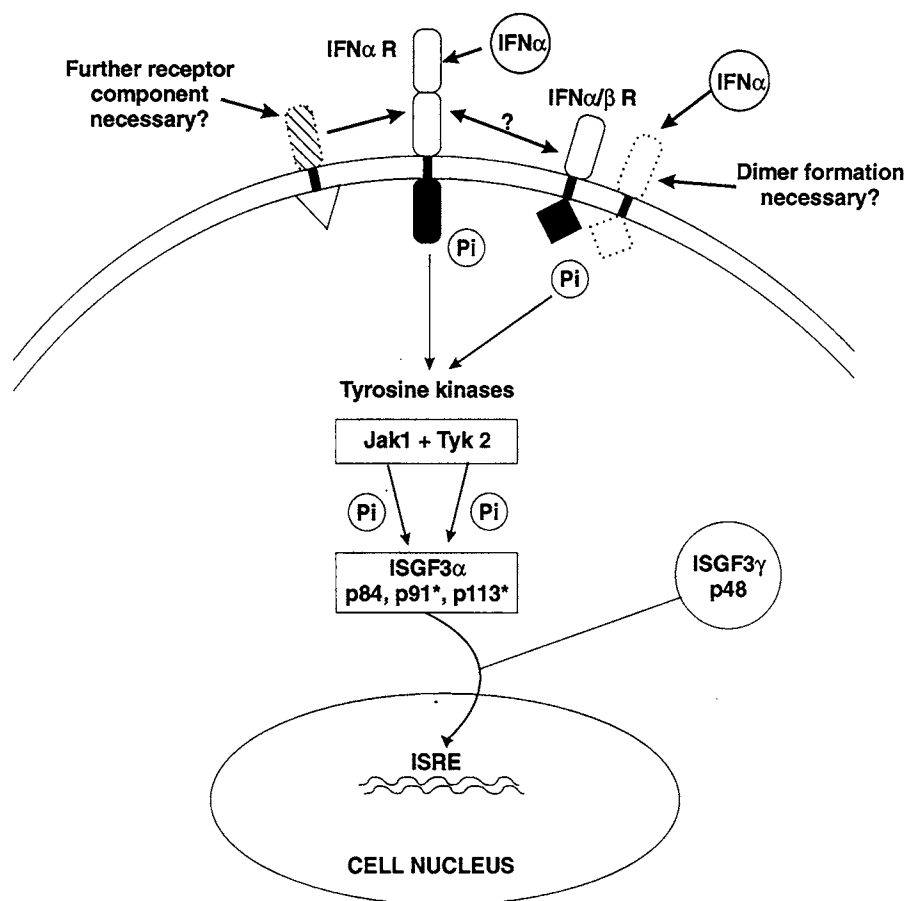
Figure 25.6   Schematic drawing depicting signal transduction pathways from the IFN receptors at the cell membrane to the cell nucleus. ISGF3 = interferon-stimulated gene factor-3. The ISGF3α complex contains three structurally related proteins p84, p91, and p113, and the ISGF3γ subunit is comprised of a single protein, p48. ISRE = interferon-stimulated response element, present in IFN-inducible genes. JAK1, JAK2 and TYK2, nonreceptor protein tyrosine kinases involved in the phosphorylation of ISGF3α proteins, p91 and p113. PKC = protein kinase C involved in the phosphorylation of nuclear transcription factor NFκB.

1, 29, 99, and 139, which are responsible for disulfide bond (Cys^1-Cys^{99}, Cys^{29}-Cys^{139}) formation in human IFN-α subtypes, are perfectly conserved. Most of the mouse IFN-α subtypes contain an N-linked glycosylation site at position 78 and thus are glycoproteins. In amino acid sequences, mouse IFN-α subtypes are about 40% homologous with their respective human counterparts (Langer and Pestka, 1985).

There is only a single-copy mouse IFN-β gene and this encodes the 161-amino-acid mature mouse IFN-β protein (Higashi et al., 1983; De Maeyer and De Maeyer-Guignard, 1988). Mouse IFN-β contains only one cysteine and thus cannot form intramolecular disulfide bonds. It has three potential N-linked glycosylation sites and is heavily glycosylated when secreted from mouse fibroblasts; the molecular mass of the native glycoprotein is approximately 34 kDa compared to the predicted 17 kDa for the nonglycosylated counterpart (De Maeyer and De Maeyer-Guignard,

1988). The amino acid sequence of mouse IFN-β is about 48% related to that of human IFN-β. The three-dimensional structure of mouse IFN-β has been solved (Senda et al., 1992) (see Section 3.1.1) and the protein has been shown to comprise five α-helices folded into a compact α-helical bundle (Figure 25.4).

Induction of transcription of mouse IFN-α subtype genes and the mouse IFN-β gene is probably regulated by transcription factor-binding nucleotide sequences present in the 5' noncoding promoter region, in a similar way to that of human IFN-α and IFN-β genes (see Section 2.2). For example, repeated GAAA-rich sequences are present in the 5' flanking regions of most mouse IFN-α subtype genes and these are likely to be important for virus-inducible transcription (Shaw et al., 1983; Zwarthoff et al., 1985). Inducers of mouse IFN-α and IFN-β synthesis, which include a number of viruses and double-stranded polynucleotides, are similar to those which induce human IFN-α, IFN-β, and IFN-ω

production (Stewart, 1979; De Maeyer and De Maeyer-Guignard, 1988). Similarly, the type of IFN produced follows the pattern found among different human cell types: fibroblastic and epithelial cell lines produce mainly IFN-β, whereas leukocytes produce mainly IFN-α subtypes (De Maeyer and De Maeyer-Guignard, 1988).

The biological properties of mouse IFN-α and IFN-β are similar to those of human IFN-α, IFN-β and IFN-ω (see Section 5). Since mouse and human IFN-α subtypes are only 40% homologous, there is considerable species preference in biological activity, i.e., mouse IFN-α is weakly active in human cells and vice versa. Mouse IFN-β is also not active in human cells (Stewart, 1979).

Rather less is known regarding receptors for mouse IFN-α and IFN-β, than for the human counterparts, but it is probable that they comprise two or more chains, as is the case for the human IFN-α/β/ω receptors (Uzé et al., 1995). The mouse equivalent receptor chain to the IFN-α_8 binding protein (Uzé et al., 1990) has been cloned (Uzé et al., 1992). The gene for this mouse IFN-α/β receptor has been located to mouse chromosome 16 (Cheng et al., 1993). The mouse IFN-α/β receptor is 564 amino acids long and is divided into a large N-terminal extracellular domain (403 amino acids), a short hydrophobic transmembrane segment (20 amino acids), and a cytoplasmic domain (141 amino acids) (Uzé et al., 1992). The extracellular domain contains eight potential N-linked glycosylation sites and is predicted to exhibit the two-D200 domain structure of the human IFN-α_8 binding protein extracellular domain (Figure 25.5) (Uzé et al., 1995). Further mouse IFN-α/β receptors or components thereof await identification and characterization. Signal transduction via mouse IFN-α/β receptors is expected to involve the JAK1/TYK2–ISGF3 (STAT 1/2) pathway as outlined for human IFN-α/β/ω receptors (see Section 7.1). In STAT 1 gene-deleted mice there are no overt developmental abnormalities, but they display a complete lack of responsiveness to mouse IFN-α and IFN-γ (Durbin et al., 1996; Meraz et al., 1996). As a consequence, STAT 1 $^{-}/^{-}$ mice are highly susceptible to infection by viruses and microbial pathogens. STAT 1 is therefore an obligatory mediator in the signal transduction pathway triggered by IFNs. Targeted disruption of the cloned mouse IFN-α/β receptor gave rise to a knockout with a similar phenotype (Müller et al., 1994). Such mice, lacking the IFN-α/β receptor, developed normally but were unable to respond to mouse IFN-α/β and thus unable to cope with viral infections.

9. Clinical Uses of IFNs

9.1 GENERAL CONSIDERATIONS

The potent antiviral activity of IFN-α/β/ω together with their potential antitumor actions provided the impetus for large-scale manufacture of IFNs for the purpose of clinical evaluation in a variety of viral and malignant diseases. In the early 1970s, IFN production depended on pooled, human buffy coats (leukocytes) and thus only limited quantities could be made (Cantell and Hirvonen, 1977). Later in that decade, human lymphoblastoid cells (e.g., Namalwa), which could be grown to large culture volumes, became available for IFN production. By the 1980s, following the cloning of IFN-α and IFN-β, these IFN species were mass-produced by recombinant rDNA technology, leading to abundant availability of certain IFN-α subtypes, e.g., IFN-α_2 and "stabilized" IFN-β ser 17. There followed production of IFN-γ and IFN-ω by this means. Clinical usage of IFN-α preparations far exceeds that of IFN-β and IFN-ω because of early production difficulties with the latter types, though these are now solved.

At the beginning of the 1980s there was tremendous enthusiasm, both from manufacturers of IFNs and from clinicians, to evaluate the therapeutic potential of IFNs. However, early clinical trials had been poorly devised, were not "blinded", and often yielded only anecdotal evidence of success. It was only after many controlled, randomized studies had been conducted that it became apparent that IFNs in general, administered as a single agent, were not beneficial for the treatment of the majority of malignant diseases, including the major cancers (lung, breast, colon) of the developed world. The initial optimism all but vanished and was replaced in the mid-to-late 1980s by a more sober and realistic appreciation of the potential therapeutic value of IFNs. A number of general conclusions have been drawn, as follows. (i) IFN-α and IFN-β, and to a lesser extent IFN-γ, have antitumor activity in a small number of cancers, particularly in those that are relatively slow-growing and well-differentiated. (ii) There is no indication that heterogeneous IFN-α preparations containing mixtures of IFN-α subtypes (e.g., leukocyte IFN, lymphoblastoid IFN) have different clinical effects from those of homogenous, recombinant IFN-α subtype or IFN-β preparations. (iii) Continuous or intermittent high dosing appears to be required for antitumor efficacy. (iv) IFNs probably work best in patients with a minimal tumor burden (Balkwill, 1989).

A major concern that has emerged from clinical studies is that IFNs all generate a considerable number of undesirable, clinically observable, side-effects, including fever, chills, malaise, myalgia, headache, fatigue, and weight loss, and in certain cases these have been severe enough for treatment to be halted (Bottomly and Toy, 1985; Rohatiner et al., 1985; Goldstein and Laszlo, 1986). In addition, a variable proportion (1–40%) of patients treated with IFN-α or IFN-β, especially recombinant IFN-α_2 and recombinant IFN-β ser 17, develop neutralizing antibodies to the IFN species used (Rinehart et al., 1986; Antonelli et al., 1991), that in some instances have been associated with clinical

"resistance" to IFN (Steis *et al.*, 1988; Öberg *et al.*, 1989; Freund *et al.*, 1989; Fossa *et al.*, 1992). A further important, but generally unrecognized, side-effect of IFN-α treatment is the possible induction of certain types of autoimmune disease (Feldmann *et al.*, 1989; Gutterman, 1994), probably mediated via IFN-induced upregulation of MHC antigen expression and generalized immunosuppression.

9.2 IFN TREATMENT OF MALIGNANT DISEASES

The most responsive cancer to IFN-α therapy is a very rare form of B-cell leukemia, known as "hairy cell" leukemia (HCL), in which a response rate up to 80% has been reported (Gutterman, 1994; Baron *et al.*, 1991; Vedantham *et al.*, 1992). In HCL patients, the "hairy cells" invade the spleen and bone marrow and the disease takes an indolent course. It has been shown convincingly that IFN-α therapy continued over several months leads to a clearance of "hairy cells" and in some patients a long-term remission is achieved. IFN-β ser 17 or IFN-γ were less effective against HCL (Saven and Piro, 1992). The IFN-α-induced mechanisms whereby clearance of "hairy cells" is achieved are not fully understood, but it is believed that a direct action of IFN-α leading to differentiation of "hairy cells" to a nonproliferating phenotype is involved (Vedantham *et al.*, 1992; Gutterman, 1994). Not all patients benefit greatly from IFN-α treatment and some develop neutralizing antibodies, particularly when IFN-α$_2$ is used (Steis *et al.*, 1988). When such neutralizing antibodies cause resistance to further IFN-α$_2$ treatment, clinical responses can be "rescued" by switching to a heterogeneous IFN-α preparation, e.g., leukocyte IFN-α (von Wussow *et al.*, 1991). However, on the whole, IFN-α therapy of HCL appears at least as effective and durable as chemotherapy with the drug pentostatin (2-deoxycoformycin) (Saven and Piro, 1992).

IFN-α therapy has also been shown to slow down the progression of chronic myelogenous leukemia (CML) (Baron *et al.*, 1991; Gutterman, 1994). In this malignant disease, leukemic cells grow slowly in the initial chronic, but benign, phase and persist for 2–4 years, but there follows a dramatic "blast crisis" producing rapidly proliferating myeloid leukemia cells and a fatal outcome. CML patients treated with IFN-α in the chronic phase often achieve durable remissions, associated with the elimination of leukemic cells bearing the so-called "Philadelphia chromosome", sometimes lasting up to 8 years.

Other malignancies in which IFN-α therapy seems to work, although with generally a lower percentage of patients responding than in HCL and CML, include low-grade non-Hodgkin lymphoma, cutaneous T cell lymphoma, carcinoid tumors, renal cell carcinoma, squamous epithelial tumors of the head and neck, multiple myeloma, and malignant melanoma. In most of these cancers, complete responses are low compared to partial responses, but IFN-α may help with maintenance therapy of diseases in some cases, e.g., multiple myeloma (Mandelli *et al.*, 1990; Johnson and Selby, 1994).

The neovascularization of primary tumors is a crucial step in their development and thus the anti-angiogenic activity of IFN-α/β (Sidky and Borden, 1987) may have therapeutic value in certain early malignancies, e.g., primary melanoma (Gutterman, 1994). Kaposi sarcoma, often found in AIDS patients, has been regarded as an angiogenic tumor or angioproliferative disease, which may explain why IFN-α treatment can lead to regression of lesions in up to 40% of patients with this condition (De Wit *et al.*, 1988; Groopman and Scadden, 1989).

In preclinical systems, the combination of IFN therapy and conventional chemotherapy has appeared to offer greater chances of producing effective treatment of many cancers, but in clinical trials this strategy has produced mostly disappointing results (see Wadler and Schwartz, 1990, for review). This may be due to (i) the inability of preclinical models accurately to predict the clinical situation; (ii) the lack of understanding of the biochemical interactions and biological consequences of combining IFNs and chemotoxic agents; (iii) a failure to incorporate information on dose, scheduling, and sequence of administration of IFNs and chemotoxic agents into clinical trials.

9.3 IFN TREATMENT OF VIRAL DISEASES

Despite having proven antiviral activity *in vitro*, IFNs have not proved the hoped-for panacea for most common viral infections in man. IFN-α/β prevent the replication of common cold viruses (rhinoviruses and coronaviruses) in the test tube and when administered to volunteers in the form of a nasal spray, but cannot "cure" colds once they are established (Scott *et al.*, 1982; R.M. Douglas *et al.*, 1986; Turner *et al.*, 1986). IFN-α is only partially effective in preventing influenza virus infections (Treanor *et al.*, 1987).

Topical applications of IFN-α/β in the form of creams or ointments to herpes virus lesions, e.g., in herpes zoster (chickenpox), and genital warts (*Condyloma acuminatum*) caused by papilloma viruses have been investigated, but have given limited beneficial effects. However, when administered parenterally, i.e., by intramuscular or intravenous injection, greater beneficial effects of IFN-α/β on virally caused lesions and warts have been found, although not to an extent that IFN therapy has become the treatment of choice (Schneider *et al.*, 1987; J.M. Douglas *et al.*, 1990; Baron *et al.*, 1991; Gutterman, 1994). Another wart-like disease, juvenile laryngeal papilloma (JLP), which can severely obstruct

the airways of young children, caused by the same papilloma virus types (6 and 11) as cause genital warts, has also been found to respond beneficially to IFN-α therapy. Disappointingly, IFN-α therapy appears neither curative nor of substantial value as an adjunctive agent in the long-term management of JLP (Healy *et al.*, 1988).

Probably the most successful application of IFN-α therapy to viral disease is in the treatment of chronic active hepatitis, caused by either hepatitis B or C viruses (Baron *et al.*, 1991; Gutterman, 1994). Up to about 40% of chronic active hepatitis B patients respond to IFN-α therapy; viral infectivity markers disappear and seroconversion and cure follow. It is interesting in the case of hepatitis B virus that viral activity is responsible for inhibiting the endogenous IFN system (Foster *et al.*, 1991), and thus the administration of exogenous IFN-α constitutes a replacement therapy. In hepatitis C virus infection, some serotypes of the virus are apparently more sensitive to IFN-α therapy than others and prolonged treatment may be necessary (>6 months) to prevent relapses occurring (Gutterman, 1994).

Both IFN-α and IFN-β have been shown to inhibit human immunodeficiency virus-1 (HIV-1) replication *in vitro* (Hartshorn *et al.*, 1987). However, *in vivo*, there is little evidence showing that IFN-α therapy has any long-term beneficial effect in asymptomatic HIV-1-positive individuals or AIDS patients (Friedland *et al.*, 1988; Lane *et al.*, 1990), except for limited regressions in Kaposi sarcoma lesions (De Wit *et al.*, 1988; Groopman and Scadden, 1989). Combination therapies for HIV-1-infected individuals involving IFN-α and antiviral drugs such as zidovudine (AZT) have also proved to be ineffective (Berglund, 1991).

9.4   IFN TREATMENT OF OTHER HUMAN DISEASES

As mentioned earlier, IFN-α/β inhibits hematopoiesis and therefore induces leukopenia in patients. This effect has generally been thought to be undesirable and it can lead to immunosuppression; however, it has proved useful for the treatment of diseases in which there is uncontrolled leukocytosis, e.g., thrombocytosis (markedly elevated platelet numbers), associated with various myeloproliferative diseases (Gisslinger *et al.*, 1989). Resistance to IFN-α$_2$ therapy has occurred in such patients when neutralizing antibodies to IFN-α$_2$ have developed, but successful retreatment with a heterogeneous IFN-α preparation, lymphoblastoid IFN (IFN-αN1) has been reported (Brand *et al.*, 1993).

The findings that production of IFN-α and IFN-γ was deficient in multiple sclerosis (MS) patients (Neighbor and Bloom, 1979) stimulated clinical trials to evaluate IFNs in MS. Rather unexpectedly, it has repeatedly been found that IFN-β, either natural fibroblast-derived or the later recombinant IFN-β ser 17 (IFN-β-1b), injected

intrathecally, subcutaneously, or intramuscularly in patients with relapsing/remitting disease leads to a reduced rate of exacerbations of the disease and thus is possibly of clinical benefit in some patients (Jacobs *et al.*, 1981, 1987, 1993; The IFNB Multiple Sclerosis Study Group, 1993; Paty *et al.*, 1993). The IFN-β-induced mechanisms that contribute to this beneficial outcome are not known, but probably immunomodulatory actions are involved, e.g., suppression of growth and activity of autoreactive T lymphocytes in the central nervous system (Goodkin, 1994). It is unclear whether IFN-α would have a similar effect. However, the results with IFN-β treatment have been encouraging so far, although more follow-up of patients will be necessary to monitor any effects on the clinical progression of MS (Ebers, 1994).

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