Section A
Molecular Aspects, Introduction and Purification

1
Type I Interferons: Genetics and Structure

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1.1
Introduction

Since their discovery about 50 years ago [1], the interferons (IFNs) have been extensively studied, they are used clinically and their multiple functions are being elucidated at the molecular level. Recent sequencing of numerous vertebrate genomes, including the human and mouse genomes, has resulted in rapid expansion of known IFN gene and promoter sequences, and detailed characterization of the IFN gene cluster. Appreciation of the diversity of the IFN gene family and its origins, coupled with progress in structural biology of IFNs, has advanced our understanding of their functions.

The type I IFN genes were first located to specific chromosomes using cytogenetic methods based on aneuploid human cells or cross-species hybrid cells. While these initial studies led to the incorrect assignment of the fibroblast IFN gene to chromosome 2 and 5 [2, 3]; subsequently, analyses of a large number of hamster–human and mouse–chimpanzee somatic cell hybrid clones led to the localization of human and chimpanzee genes encoding IFN-β onto chromosome 9 [4]. During the same period, the unusual absence of introns within genes encoding IFN-α and -β was discovered by two separate groups [5, 6]. Leukocyte and fibroblast IFN cDNA clones were used to generate radioactive probes for hybridization experiments on human cells and human–mouse hybrid cells. These enabled the identification of multiple *IFNA* genes and a single *IFNB* gene on human chromosome 9 [7]. *IFNA* and *IFNB* genes were then mapped onto chromosome 9p and blot hybridization detected a cluster of at least 10 genes encoding IFN-α subtypes and a single gene encoding IFN-β on chromosome 9p ter-q12 [8]. Multiple functional genes and pseudogenes encoding IFN-α and -ω, and a single gene encoding IFN-β were identified in this region, and their relative position and transcriptional directions determined [9, 10]. During this time, the existence of multiple IFN-α subtypes was also discovered at the protein level [11]. Complete sequencing, extensive analysis and annotation of human chromosome 9 was completed recently, and the human type I IFN gene cluster was extensively characterized [12, 13].

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IFNs are grouped into three separate classes: types I (α/β IFNs, etc.), II (IFN γ) or III (λ IFNs), based on their sequence, receptor specificity, chromosomal location, physicochemical properties and structure. Type I IFNs are unique among the IFN family with unusual physicochemical properties of heat (65 °C) and acid stability (pH 2) [14]. Originally, type I IFNs were named leukocyte IFN (IFN-α) and fibroblast IFN (IFN-β) based on the perceived cellular origins of these cytokines. However, it is now accepted that most nucleated cells are able to produce IFN [15, 16], but specialized IFN-producing cells (IPC) such as plasmacytoid dendritic cells can produce 1000-fold higher levels of IFN when stimulated by an appropriate inducer [17]. Microorganisms such as viruses, bacteria, mycoplasma, protozoa, their products such as viral glycoprotein, bacterial lipopolysaccharide (LPS), CpG DNA, double-stranded RNA, host-derived molecules such as mitogens, other cytokines and a variety of other stimuli induce type I IFN production [18, 19].

In addition to IFN-α and -β, other members of the type I IFN gene family, such as IFN-ε and -κ are present in most mammalian genomes, while IFN-ω is present in primate and some mammalian genomes, and IFN-ζ and -ν are only present in rodent and feline genomes, respectively [20, 21]. Other type I IFNs such as IFN-τ and -δ that are involved in reproduction events have been identified, and are unique to ungulate ruminants and pigs [22–24]. This entire repertoire of type I IFNs is believed to bind to and signal through the cell surface IFNAR receptor complex, consisting of IFNAR-1 and -2 transmembrane receptor chains. However, binding of IFN-κ, -ε and -ν to the IFNAR receptors has not been determined. The genes encoding other types of IFN are located on different chromosomes and bind different receptor complexes.

1.2 The Type I IFN Genetic Locus

The human type I IFNs are encoded by a multigene family clustered over a 350-kb region on human chromosome 9p21 [10, 12, 13, 25, 26]. This gene family consists of 13 nonallelic IFN-α genes, at least five pseudogenes, and single functional IFN-β, -ε, -κ and -ω genes. Analysis of public sequence databases [27, 28] shows that almost all type I human IFNs are clustered together on chromosome 9 (9p21.1–9p21.2) between *IFNB1* and *IFNE* genes, which are positioned towards the telomere and centromere, respectively (Fig. 1.1A). The exception is *IFNK*, which is located approximately 6.4 Mb from *IFNE* towards the centromere and is transcribed towards the centromere. Within the cluster, genes are located in the following order: *IFNB1*, *IFNW1*, *IFNA21*, *IFNWP15* (pseudogene), *IFNA4*, *IFNWP9* (pseudogene), *IFNA7*, *IFNA10*, *IFNAL* (pseudogene), *IFNWP18* (pseudogene), *IFNA16*, *IFNA17*, *IFNWP5* (pseudogene), *IFNA14*, *IFNAP22* (pseudogene), *IFNA5*, *KLHL9* (kelch-like protein 9), *IFNWP20* (pseudogene), *IFNA6*, *IFNA13* and *IFNA2*; all of which are transcribed in the same direction (towards the telomere). *IFNA8*,

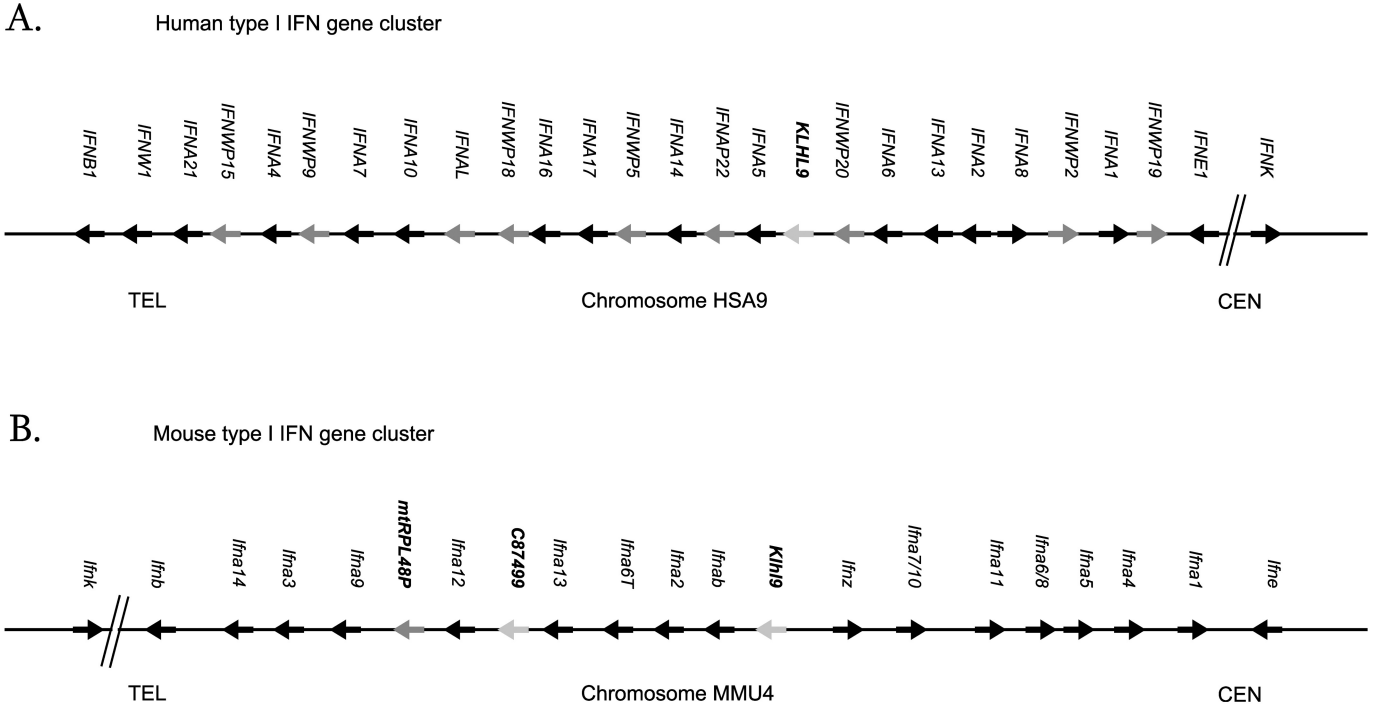

Fig. 1.1. The human and murine type I IFN gene locus. (A) Human IFN gene cluster on chromosome 9 (HSA9). (B) Murine IFN gene cluster on chromosome 4 (MMU4). The IFN gene locus was drawn using current genomic information available on NCBI human chromosome 9 (HSA9) contig (contig ID; NT_008413.16) and mouse chromosome 4 (MMU) contig (contig ID; NT_039260.4). Arrows indicate relative position and transcriptional orientation of genes. Black arrows indicate functional IFN genes, dark grey arrows pseudogenes and light grey arrows other non-IFN genes within the cluster. Orientation of the chromosome is indicated by TEL (telomeric end) and CEN (centromeric end). Nomenclature suggested by van Pesch et al. [32] is used in naming murine IFNs. (This figure also appears with the color plates.)

IFNWP2 (pseudogene), *IFNA1* and *IFNWP19* (pseudogene) are transcribed in the opposite orientation (towards the centromere), with *IFNE* transcribed towards the telomere. The relative position of IFN genes within the gene cluster and their transcription orientations is shown in Fig. 1.1(A). The only non-IFN gene within the human cluster, *KLHL9*, is syntenic on human and mouse chromosome HSA9 and MMU4, respectively, and is a homolog of the *Drosophila kelch* gene. These are implicated in embryogenesis, tissue development and carcinogenesis, and also mediate cytoskeleton organization [29, 30].

Murine type I *Ifn* genes were mapped to a centromere proximal region on (42.6 cM) *Mus musculus* (MMU) chromosome 4, and almost all of these *Ifn* genes are clustered on a 450-kb region [31] (Fig. 1B). The overall order of genes in the type I *Ifn* locus is conserved between human and mouse genomes. This region contains at least three pseudogenes, 14 functional *Ifna* genes, and single *Ifnb*, *Ifnk*, *Ifne* and *Ifnz* (limitin) genes. Due to the low level of sequence resolution and the possibility of assembly artifacts in the *Ifnz* region, the exact number of *Ifnz* genes is not known. In a recent study, Hardy et al. [13] reported the presence of at least two genes and van Pesch et al. [32] detected a tandem array of 16 consecutive, almost identical *Ifnz* genes on the National Center for Biotechnology Information (NCBI) contig assembly. Within the main cluster of IFN genes, *Ifnb* is located more towards the telomere, whereas *Ifne* is located more towards the centromere. Murine *Ifnk* is located approximately 52 Mbp distal to *Ifnb* and the main IFN gene cluster, and is transcribed towards the centromere. Other IFN genes are located between these *Ifnb* and *Ifne* genes in the following order: *Ifnb*, *Ifna14*, *Ifna3*, *Ifna9*, *Ifna12*, *Ifna13*, *Ifna6T*, *Ifna7/10*, *Ifna2* and *Ifnab*; all transcribed in a single direction with their 3′-end towards the telomere (Fig. 1B). *Ifnz*, *Ifna7/10*, *Ifna11*, *Ifna6/8*, *Ifna5*, *Ifna4*, *Ifna1* and *Ifne1* form the remainder of the IFN gene cluster, and except for *Ifne* these genes are arranged in tandem in the opposite orientation and transcribed towards the centromere. Three non-IFN genes are located within the *Ifn* gene cluster and include the *klhl9* gene as in the human genome.

Interestingly, mammalian and avian type I IFNs lack introns – an unusual property shared with a number of other genes such as those that encode histones and G-protein-coupled receptors [33]. Recent genome analysis indicates that intronless genes are more common in human and other eukaryotic genomes than previously believed, with at least 12% of human genes being intronless [34–37]. The absence of introns in IFN genes may have been a property of the common ancestral gene or may have resulted from retro-transposed copying of an-intron encoded gene. The existence of introns in fish IFNs and in distantly related IFN-λ genes, and the presence of a single intron in the 3′-untranslated region (UTR) of IFN-κ suggests that IFN genes may have lost their introns due to retro-transposition. Their intronless gene structure and chromosomal colocalization, together with the multiplicity of IFN genes and extensive sequence conservation, indicates that this gene family arose by gene duplication. A similar expansion of genes encoding IFN-α is found in genomes of other mammals and in avian genomes, supporting the biological importance of this gene family.

1.3
Type I IFN Genes

1.3.1
IFN-α

The 13 human *IFNA* genes encode 12 different IFN-α proteins with *IFNA1* and *IFNA13* encoding an identical mature protein [26]. The IFN-α proteins share 76–99% amino acid identity and include a hydrophobic, 23-amino-acid signal peptide plus a 166-amino-acid mature peptide sequence. The exception is IFN-α2, which has a deletion at position 44 and encodes a 165-amino-acid protein. In addition, some of these subtypes exist in variant polymorphic forms such as IFN-α2a (K23, H34), -2b (R23, H34) and -2c (R23, R34) [38].

When murine *Ifna* gene sequences submitted to public databases were compared to the genomic sequence, discrepancies in nomenclature of some *Ifna* genes were identified. Certain *Ifna* genes such as *Ifna6* and *Ifna8* were identified as the same gene, as were *Ifna7* and *Ifna10* [32]. Therefore, these were named *Ifna6/8* and *Ifna7/10*. Most corresponding murine *Ifna* genes from different mouse strains such as 129/Sv, C57BL/6 and BALB/c showed 99% sequence identity; however, interestingly, genes encoding IFN-α6/8, -α7/10, -α11 and -α1 diverge substantially between mouse strains [32]. Except for *Ifna1*, the divergent genes all cluster together, suggesting that even though these may have originated from a cluster of common ancestral genes, they were subjected to independent evolution in separate mouse strains [32]. The IFN-α subtypes are produced in response to infection by diverse microorganisms and represent a first-line defense, particularly in viral infections. Individual subtypes are differentially expressed depending on the inducer and the producing cell [18, 39].

1.3.2
IFN-β

Unlike genes encoding IFN-α, duplication and expansion of the *IFNB* gene has not occurred in most mammalian genomes. A single gene encoding IFN-β is present in most mammalian genomes with the exceptions of ruminant (bovine, equine) and porcine genomes, where more than one copy of this gene is present. In most species extensively examined so far, only a single *IFNB* gene is present, although in a study of 25 Caucasian families, the *IFNB* gene segregated as a single copy, but duplication of the *IFNB* gene was seen in some members of two of the families and is believed to be a recent event [39].

Human IFN-β consists of 166 amino acids and shows 25–32% identity to human IFN-αs, whereas murine IFN-β consists of a 161-amino-acid mature protein and displays only 19–23% identity to murine IFN-αs [26]. IFN-β is reportedly expressed

during myeloid cell differentiation and has a function there. Otherwise, it is produced like IFN-αs in response to infection, notably in response to Gram-negative bacteria or their constituent LPS, which interestingly induces no IFN-α [40].

1.3.3 IFN-ω

IFN-ω is a type I IFN that is distinct from α and β IFNs [41]. There are multiple *IFNW* genes in the human genome, but only one of them is a functional gene, while the rest are pseudogenes [42]. Orthologs of IFN-ω have been identified in the genomes of cattle, sheep and horses, but not in mice [43, 44].

The mature human IFN-ω consists of 172- and 174-amino-acid polypeptides, the latter being a minor species generated by alternative signal peptide cleavage. Similar to all the other IFNs, this subtype signals through the IFNAR receptor complex [42, 45]. These proteins are *N*-glycosylated at a position (Asn78) corresponding to human IFN-β and murine IFN-α, but are more related to α subtype IFNs with 55–60% identity [46]. IFN-ω is produced in response to viral infection, like IFN-α subtypes [47].

1.3.4 IFN-κ

The gene encoding IFN-κ is located at least 60 Mb telomeric from the rest of the human IFN gene cluster and its isolation from the rest of the cluster suggests separate evolution. This gene may have diverged early to play a specific role in mammals. Mouse, rat and chimpanzee counterparts of the *Ifnk* gene show a similar solitary location. While the mammalian type I IFN genes usually lack introns, the *IFNK* gene is the exception because it has a single intron in the 3′-UTR immediately following the stop codon. This may be important in the transcriptional regulation of this gene.

The single human IFNK does not show preferential identity with any other single IFN subtype, having only 27–32% identity to IFN-α subtypes, 34% identity to IFN-β, and 28% identity to IFN-ω and -ε. This type I IFN encodes a 180-amino-acid protein which is slightly larger than other IFNs due to an insertion in the CD loop.

IFN-κ is constitutively expressed by keratinocytes and other cells of the innate immune system, such as monocytes and dendritic cells, and shows some similarities to IFN-β [48] in binding to heparin with a high affinity. This binding may assist in maintaining higher local concentrations of IFN-κ [49].

1.3.5
IFN-ε

A single IFN-ε-encoding gene is present in a syntenic region of mouse, rat and human genomes. This *IFNE* gene is located in the extreme centromeric region of the human IFN gene cluster on 9p21 and the protein encoded by this gene is more structurally related to IFN-β than any other type I IFNs [13]. The mature protein is a 185-amino-acid polypeptide and contains a C-terminal extension relative to other type I IFNs. The human IFN-ε polypeptide is 15 amino acids longer than mouse IFN-ε, and shares 54% amino acid sequence identity and 69% similarity [13]. Murine and human IFN-ε possess reproductive hormone-regulatory elements on their promoter sequence, and are constitutively expressed in murine placental and ovarian tissue, indicating a possible reproductive role in placental mammals, perhaps similar to IFN-τ in ungulate ruminants [13].

1.3.6
IFN-ζ (Limitin)

Another IFN-like cytokine was recently identified in the mouse IFN gene cluster [50, 51]. Due to its growth-limiting ability it was named limitin; however, further analysis suggested that this protein belonged to the type I IFN family and led to this protein being named IFN-ζ [52]. The exact number of *Ifnz* genes in the mouse genome is undetermined, no homologs have been identified in humans and only an *Ifnz* pseudogene is present in the rat genome. The *Ifnz* gene consists of two potential open reading frames that can encode two different proteins, but there is preferential use the second ATG that encodes a full-length biologically active cytokine.

This IFN subtype encodes a 21-amino-acid signal peptide and a 182-amino-acid mature protein, with a single *N*-glycosylation site at Asn68. It has 25–28% amino acid identity to IFN-α, 21% identity to IFN-β and 30% identity to IFN-ω. Similar to IFN-β, this IFN is also a heparin-binding protein [51, 53]. This protein is constitutively produced, has potent B lymphopoiesis activity and shows a limited expression profile [53]. IFN-ζ has very high antiviral, immunomodulatory and antitumor activity compared to other IFN-αs, and it does not cause fever or myelosuppression, which are common clinical side-effects of other type I IFNs [54]. Its unique properties indicate that it may be useful as a novel therapeutic without toxic side-effects common to other IFNs.

1.3.7
IFN-τ

In addition to the IFNs commonly seen in murine and primate organisms, IFN-τ is a pregnancy-related IFN found only in ungulate ruminants (such as sheep, cat-

tle, goats, musk ox, red deer, giraffe, etc.). Multiple genes encoding IFN-τ are seen in ruminant genomes. This unique physiological IFN is believed to have evolved by duplication from an *Ifnw* gene which acquired a promoter region that imparted trophoectoderm specific expression. Phylogenetic analysis indicates that this duplication event occurred about 36 million years ago (MYA) [55]. This protein is believed to be involved in the maternal recognition of pregnancy by prolonging the lifespan of the corpus luteum [56]. Unlike other IFNs, IFN-τ is not induced by viruses and is constitutively expressed by the embryo, with maximal expression detected prior to implantation [56]. Similar to IFN-ω, these IFNs contain a 6-amino-acid extension at the C-terminus. Even though IFN-τ genes have a high degree of conservation, sequence identity within a species is greater than between species, suggesting continued independent duplication of genes in different lineages of ruminants [57]. In bovine species these genes are clustered close to IFN-α and -ω genes on chromosome 8, which is syntenic to human chromosome 9 and mouse chromosome 4.

1.3.8
IFN-δ

IFN-δ is another pregnancy-related IFN subtype which was identified in pigs and encodes a 149-amino-acid protein. The porcine genome encodes two intronless nonallelic loci with strong homology to IFN-δ cDNA, one of these was a pseudogene with a premature stop codon and the other encoded the IFN-δ protein. The 5′ promoter region of this gene is devoid of regulatory and transcription factor binding elements needed for virus inducibility, consistent with the lack of viral inducibility of this gene. It has no preferential homology to other IFN subtypes with only 42% identity with human IFN-α2 and 27% identity to murine IFN-β [24]. IFN-δ is more divergent than any other IFNs and shows very little homology to the ruminant trophoblast IFNs. The gene encoding IFN-δ is believed to have diverged from the common ancestral sequence 180 MYA and before the mammalian radiation [24]. There are no human or murine orthologs of IFN-δ. Due to its shorter length than other IFNs it was originally called spI IFN (short porcine type I). The reduced length of the molecule is due to a 7-amino-acid deletion in the C-terminal end. This central deletion occurs in a region with the highest variability among the different IFNs and corresponds to a loop region.

1.3.9
IFN-ν

IFN-ν is a novel type I IFN subtype recently described by Krause and Pestka [21]. Homologs of this gene are present in most eutherian mammals such as cat, mouse, human, dog, pig, olive baboon and chimpanzee. However, only the feline genomes encode a functional *Ifnv* gene, while in all other sequenced mammalian

genomes *Ifnv* is a pseudogene [21]. This gene is located about 25 kb downstream of IFN-β and is transcribed in the same orientation as IFN-β [21]. The function of this gene in cats is yet to be elucidated.

1.4 Type I IFN Gene-regulatory Regions

As mentioned above, the individual type I IFNs show differences in both "constitutive" and inducible expression, and therefore different functions associated with when and where they are produced. Since the type I IFNs are considered the first line of defense against infections, they require rapid and controlled regulation of gene expression. Comparative global genome analyses by programs such as AVID and VISTA [58, 59] have been effectively used to identify regulatory regions based on sequence homology across species. Such analyses of the 5′ sequences in type I IFN genes indicates that the predicted regulatory promoter region of all these genes resides within 500 bp upstream of the ATG (exemplified by the IFN-β genes of human, mouse, rat dog and cow in Fig. 1.2A).

The 5′ promoter region of type I IFN subtypes shows a high degree of sequence identity (Fig. 1.2B) and conserved transcription factor binding motifs in most mammalian IFN promoters are consistent with their common functions. In the 5′ putative promoter region of eutherian mammals, multiple copies of GAAA tetranucleotide elements are present usually preceded by a spacer or AA dinucleotide elements and form part of the core IFN-regulatory factor (IRF) binding sites. A number of different transcription factors binding sites such as nuclear factor (NF)-κB, Jun, ATF and IRFs are present in type I IFN promoters, and the activation of these transcription factors during viral infection has been studied in detail.

The IFN-β promoter has been well characterized and serves as a paradigm for the study of transcriptional control of gene expression [60]. The regulatory sequences that form the IFN-β promoter are located within a 110-bp region immediately 5′ of the transcription initiation start site [61, 62]. Nucleosome remodeling occurs in this promoter region prior to transcription [63]. Virus inducibility of IFNs is conferred by a virus specific enhancer region known as the virus response element (VRE), present in the promoters of both IFN-α and -β genes (Fig. 1.2B). Within the VRE of IFN-β there are four positive regulatory domains (PRDs) designated PRDI–IV to which transcription factors bind during virus-induced gene induction [64–66]. A negative regulatory element is also located in the region partially overlapping PRDII. Transcription factors such as IRF-1, -2 and -3 bind to PRDI, NF-κB binds to PRDII, IRF-3 binds to PRDIII, and activating transcription factor (ATF)-2/c-Jun binds to PRDIV [67, 68]. Within this promoter region, there are two binding sites within the minor groove of AT-rich sequences for the high-mobility-group protein HMGI(Y) – one near the ATF-2/c-Jun site and the other near the NF-κB site [69, 70]. These PRD regions interact synergistically to activate IFN-β gene transcription.

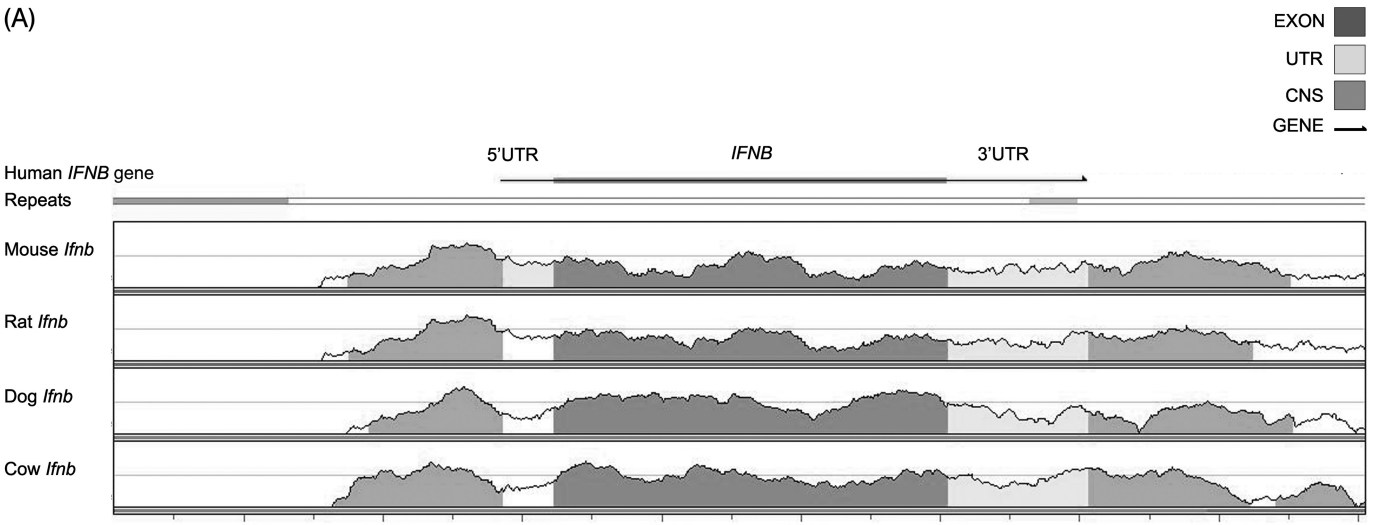

Fig. 1.2. Human type I IFN promoter sequence alignment. (A) VISTA alignment of human, mouse, rat, dog and cow IFN-β genes. The figure represents an AVID genome alignment [58] and VISTA visualization [59] of mouse, rat, dog and cow *Ifnb* genomic sequences compared to the human *IFNB* genomic sequence. At the top, the human *IFNB* gene is depicted by a dark blue line with an arrow indicating transcriptional orientation, together with 5′- and 3′-UTR regions depicted as light blue lines. The line immediately below indicates the location of repetitive elements within the genomic sequence. The graphed regions below demonstrate homology of mouse, rat, dog and cow *Ifnb* genomic regions to the human *IFNB* gene. Exon regions with more than 65% sequence identity over 100 bp are indicated by dark blue, whereas conserved noncoding sequences (CNS) and 5′- and 3′-UTRs with over 65% sequence identity are depicted in orange and light blue, respectively.

In addition to the similarities in the regulatory regions of IFN genes, there are also differences and these sequence differences are likely to explain the differences in expression of particular type I IFN genes in different circumstances. Transcription factors such as NF-κB and IRF-3 are involved in inducing IFN-β transcriptional activity in infected cells, and IRF-3 and -7 are important for IFN-α transcription. Accordingly, NF-κB sites are found in the promoters of IFN-β, -ε and -α14 genes only. Unlike the IFNB promoter, the IFNA VRE does not have NF-κB sites, but contains several PRDI-like elements (Fig. 1.2B). The VRE of IFN-α1 and -α4 also contains binding sites for IRF-1, TG protein and αF-1 binding proteins. Cooperation of these factors is necessary for efficient virus-mediated induction of IFN-α. Selective binding of transcription factors to different regulatory sequences within the IFN-α and -β promoters determines the specificity of induction of these cytokines. Even though there is a high degree of sequence conservation among the 5′ promoter regions of human *IFNA* genes, due the presence of different types of transcription factor-binding elements, and the location and number of these elements result in differences between these IFN promoters. Similarly, the differences in promoter sequences among the type I IFNs will enable the binding of different transcriptional regulators according to the cell, stimulus or mechanism of activation.

In addition to regulatory elements seen in promoters, other elements within IFN genes are also important in regulation of IFN gene expression. The AU-rich elements present in the 3′-UTR of mammalian IFN genes and *cis*-acting CRID (coding region instability determinant) elements in transcripts encoding IFN-β are implicated in rapid mRNA turnover [71, 72].

1.5 Evolution of the Type I IFNs

1.5.1 Vertebrate IFN Genes

IFN genes are present in all vertebrates and form an evolutionarily conserved critical component of the host defense system [73]. Even though downstream mediators of IFN signaling such as STAT (signal transducers and activators of transcription) proteins have been identified in invertebrates [74], no IFN genes have been identified in these organisms. A report stating the ability of recombinant feline IFN-ω to mediate antiviral effects on Japanese pearl oysters (*Pinctada fucata martensii*) infected with akoya virus [75] and bind to receptors on hemocytes from these oysters presents interesting possibilities of IFN-like systems existing in invertebrates [76].

Comparison of human and mouse IFN genes and their promoters show large regions of conservation, indicating some expansion of the type I IFN gene cluster occurred before the divergence of mouse and human from a common mammalian

(B)

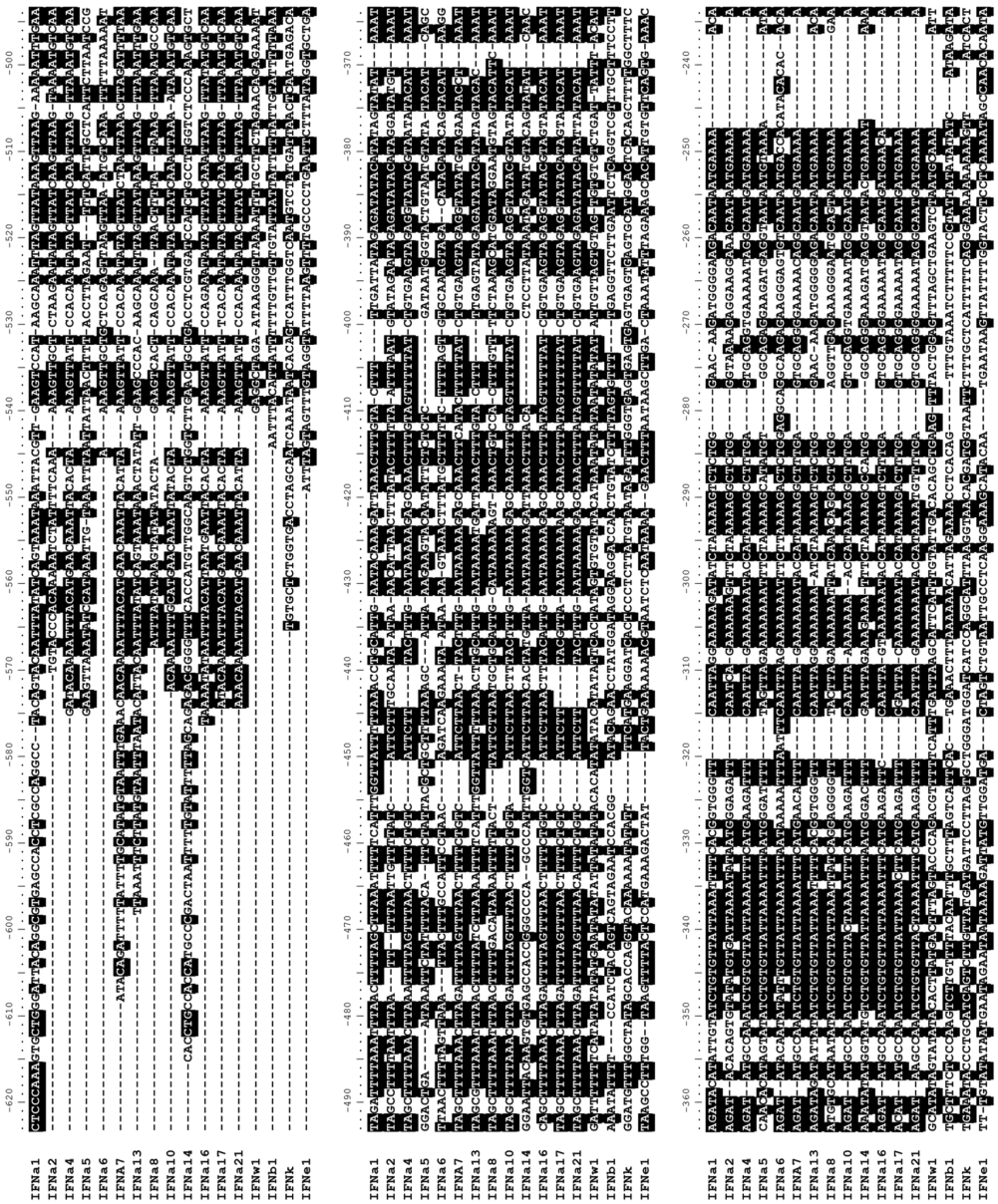

Fig. 1.2. (B) Nucleotide sequence alignment of human IFN promoter. Alignment was performed using ClustalW algorithm [79] of 500 bp 5′-flanking sequences containing the promoter elements of human IFN genes. The regulatory elements present in this region are indicated by boxed regions. (This figure also appears with the color plates.)

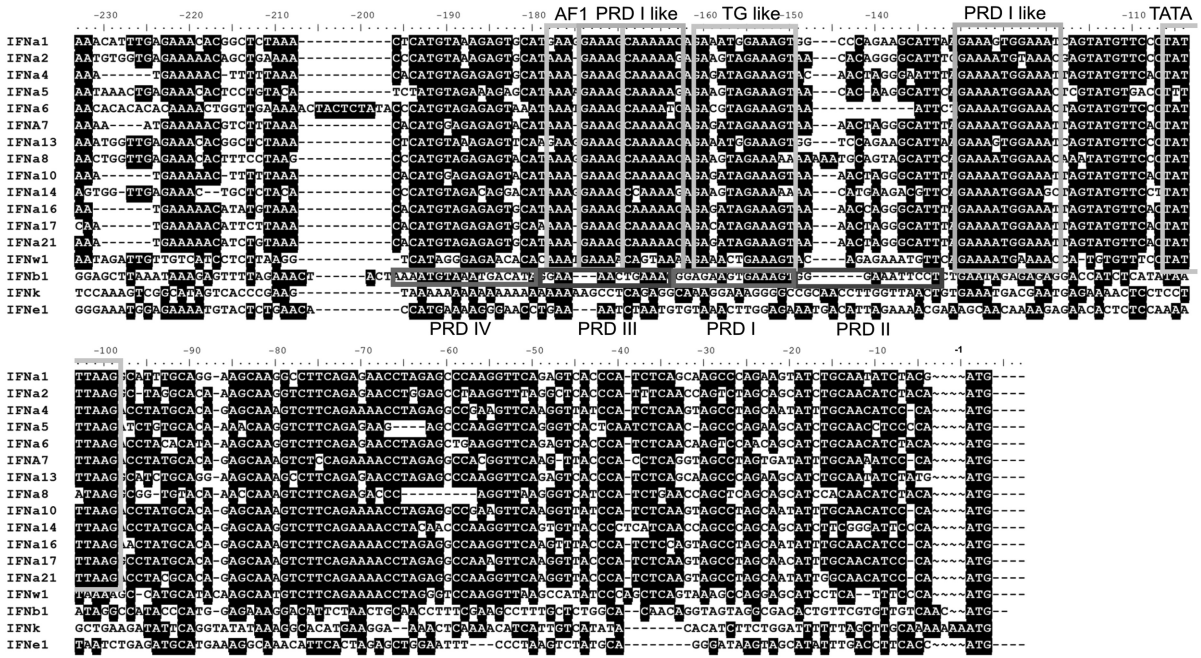

Fig. 1.2. (*continued*)

ancestor [12]. The type I IFN systems of different mammalian species show distinct similarities, with most mammals having multiple genes encoding IFN-α, and a single gene encoding IFN-β and most other type I IFNs. A small number of *Ifnb* gene duplications have been observed in ruminants. The three main type I IFN subfamilies, IFN-α, -β and -ω, are believed to have diverged after the mammalian–avian separation, but before the radiation of the eutherian mammals [77]. Phylogenetic analysis of mammalian, bird and fish IFNs demonstrates the clustering of similar subtypes from different species (Fig. 1.3). Phylogenetic analysis also suggests that genes encoding IFN-κ, -β and -ε may have diverged first from the ancestral IFN gene [57, 78, 79]. IFN-ω is believed to have diverged about 120 MYA from IFN-α, and is represented as multiple genes in some species and appears to have been lost in others [57]. The genes encoding IFN-τ have unique physiological functions involved in pregnancy and are believed to have originated from IFN-ω, with multiple copies of IFN-τ seen in some ungulate ruminants [55]. Only a single functional IFN-δ gene is present in porcine genomes and its progenitor may have pre-dated mammals.

Multiple subtypes of IFN-α appear to diverge after speciation, and it is notable that particular subtypes such as IFN-δ, -ξ, -τ and -ν found in pigs, rodents, ruminants and cats do not have individual subtypes in other species (except very closely related species, like IFN-τ within ruminants). Thus, once a species forms, it appears to be important to evolve multiple subtypes of IFN-αs. The reason for this is likely to be due to both different functions carried out by individual subtypes and to ensure some are produced in response to a broad range of pathogens. Numerous mammalian IFNs have been cloned and sequenced, and demonstrate the homology of these proteins in diverse species. A recent report of cloning and expression of five feline type I IFN genes demonstrates the similarity of these proteins even among distantly related mammals. Feline IFN-α1, -α2, -α3 and -α6 have very high sequence identity to each other, and these IFN sequences show 60% identity to human IFN-α2 and approximately 50% identity to human IFN-ω. Feline IFN-α5 is similar to feline IFN-ω due to the presence of an additional 5 amino acids inserted at position 139 of the sequence [80, 81]. Similarly, equine IFN-α subtypes showed 71–77% identity to human IFN-αs, while the equine IFN-β subtype showed 55% identity to the human ortholog [44].

Genomic information from marsupial and monotreme species, together with that from eutherian mammals, allows comparison of molecular evolution and IFN gene function in mammals with a relatively recent common ancestry. The type I IFNs of marsupial and monotreme species are classed into group I and II based on sequence identity. Analysis of the tammar wallaby *Macropus eugenii* genome demonstrated that both IFN-α and -β are present in this species [82]. Marsupial and monotreme IFN sequences also demonstrated that tammar wallaby group II and echidna (*Tachyglossus aculeatus*) group I IFNs contain the conserved Cys99 residue seen in eutherian (placental) mammals [82–84]. The wallaby group II IFNs are similar to the eutherian IFN-α and these IFNs also contain a complete set of conserved cysteine residues. Analysis of monotreme IFN genes in echidna resulted in the identification of two distinct groups of IFNs – three group I genes

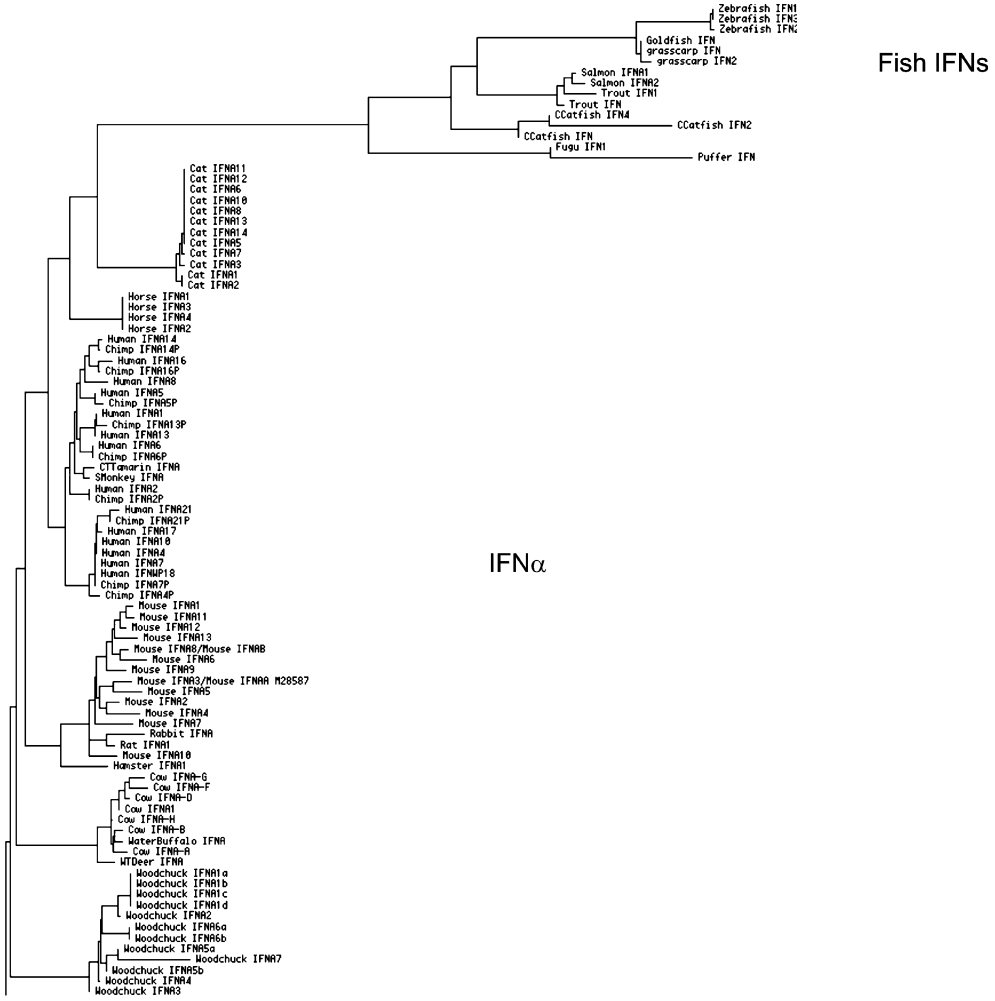

Fig. 1.3. Phylogenetic tree of vertebrate type I IFNs. The IFN amino acid sequences were aligned using the ClustalW algorithm [79], and the phylogenetic tree was constructed by calculating the distances between sequences using nongapped positions and corrected for multiple substitutions using the Kimura correction.

and a single group II gene [84]. Interestingly, NF-κB sites are present in the wallaby group I IFNs and human/murine IFN-β promoters, and similar to promoters of eutherian IFN-α genes wallaby IFN group II promoters lack NF-κB-binding sites. These putative, wallaby IFN promoter regions also contain GAAA-rich elements that are core binding sites for IRFs and related regulatory proteins. These regulatory regions are similar to elements seen in promoters of eutherian IFNs. Full-length IFN sequences, cysteine profiles and 5′ promoter sequences from

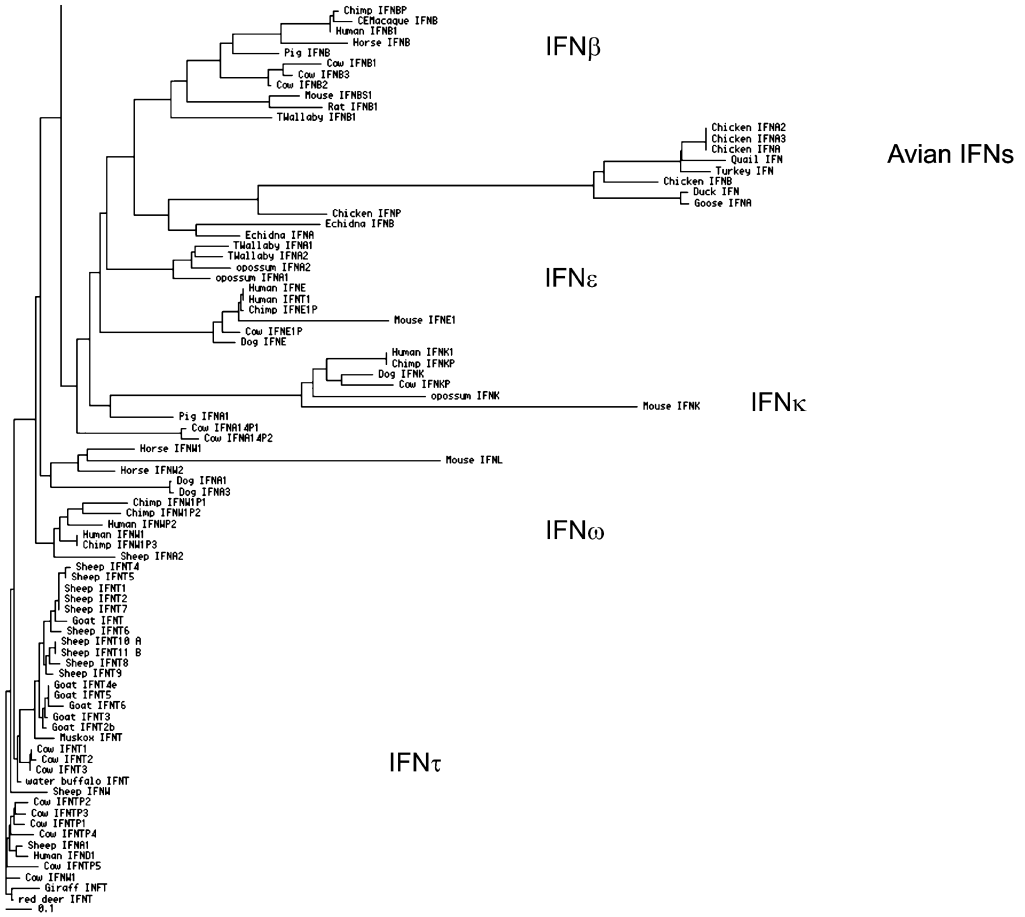

Fig. 1.3. (*continued*)

echidna also demonstrate that echidna group I IFN genes are more similar to those encoding primate IFN-α and the group II gene is similar to IFN-β [84]. Thus, placental mammals, marsupials and monotremes have evolved similar IFN systems.

Cloning nonmammalian vertebrate IFN genes was initially difficult because of weak sequence homology with their mammalian counterparts. However, the sequencing of multiple vertebrate genomes has yielded a large amount of sequence information on nonmammalian IFNs during the last few years. Recent outbreaks of avian flu and swine flu, and the impact on the poultry industry and farming, together with viral infections in aquaculture farming and implications of viral diseases of avian origin such as SARS (severe acute respiratory syndrome) and influenza on human health have rekindled interest in avian, porcine and fish IFNs.

The first nonmammalian IFN was cloned from virus-infected chick embryo

cDNA [85], and chicken IFN was purified from embryonated chicken eggs and chick embryo cells [86–88]. This protein was shown to be a glycosylated, acid- and heat-stable protein of 20–30 kDa. Avian type I IFNs are classified as IFN I (α-like) and IFN II (β-like) genes [89]. Unlike mammals, which have their IFN genes located on autosomes, birds have type I IFN genes on their sex chromosomes. Comparison of the completed genome sequences revealed extensive conserved synteny between parts of human chromosome 9 (which contain the human IFN gene cluster) and chicken sex-determining chromosome Z, with 17 of the 24 known chicken Z chromosomal genes having orthologs in chromosome 9 [12]. Interestingly, the chromosomal region close to the IFN gene cluster on human chromosome 9 contains genes involved in testicular differentiation and hemizygous deletion of this region results in human XY sex reversal, suggesting parts of chromosome 9 may have been derived from sex chromosomes of a common ancestor of mammals and birds in the evolutionary past [12]. Cluster of about 10 IFN-α-like genes and a single IFN-β-like gene, all of which are intronless, were identified on the short arms of chicken and long arm of duck Z chromosomes [90, 91]. Induction of IFN-like activity in other avian species such as turkey, pheasant, bobwhite quail, guinea fowl and duck has been reported, and IFN genes from numerous bird species have been cloned [92–94], indicating the presence of these genes in many avian species. Even though there is no detectable sequence homology between mammalian and avian IFN promoter sequences [78], genome analysis identified specific similarities between avian IFN-α-like gene promoters, with promoters of mammalian IFN-α subtypes. These IFN-α-like genes lack the NF-κB transcription factor binding elements that are found in mammalian IFN-β and avian IFN-β-like gene promoter regions [94, 95]. However, phylogenetic analysis supports the hypothesis that the gene duplication event that gave rise to mammalian IFN-α and -β may have occurred after the last common ancestor of birds and mammals diverged. Therefore, the avian and mammalian IFN-α and -β subtypes are not orthologs, and may have evolved independently from a common ancestor in mammals and birds to give rise to similar subtype functionality [78].

For many years IFN-like antiviral activity has been observed in virally infected fish and fish cell lines [96]. The existence of IFN-stimulated genes (ISGs) and other downstream mediators of IFN activity were identified in a number of fish species. Homologs of *Mx*, *Vig1*, *Vig2*, *IRFs*, *PKR* and *ISG15* genes were identified in fish, and a transcriptome analysis of rainbow trout (*Oncorhynchus mykiss*) revealed that IFN stimulated genes make a major contribution to response against a rhabdovirus infection [97, 98]. Interestingly, the promoter of the single fugu *Mx* gene can be induced by human IFN, when transfected into human cells [99]. The first fish IFN gene was identified by searching a zebrafish (*Danio rerio*) expressed sequence tag (EST) database [98]. This fish IFN cDNA encoded a 185-amino-acid protein with a hydrophobic 22-amino-acid signal peptide. These proteins display only about 15% sequence identity to mammalian and avian IFNs, and unlike the acidic mammalian and avian IFN proteins the fish proteins are highly basic [98]. Later this zebrafish IFN sequence was used to uncover an IFN gene in puffer fish (*Fugu rubripes*) [98], and similar genes have been cloned from channel catfish (*Icta-*

lurus punctatus) [100] and Atlantic salmon (*Salmio salar*) [101]. Unlike in mammals, the presence of introns is characteristic of fish IFNs, with both Atlantic salmon and zebrafish IFNs having a gene structure of five exons and four introns [101]. Another IFN gene (*TnIFN*), which is structurally related to type I and λ IFNs, was cloned from *Tetraodon nigroviridis*. This *TnIFN* has four introns similar to the λ IFNs, and was shown to induce *Mx* and *PKR* genes [102]. Information on IFN genes in amphibian species is extremely rare. However, *xIRF6*, which is an IRF-related gene, expressed during early developmental stage of *Xenopus laevis* has been cloned [103]. Existence of reptilian IFNs has been inferred from antiviral activity mediated by acid-stable, heat-resistant 33-kDa proteins produced by turtle and tortoise (*Testudo graeca*) cells infected with virus or stimulated with IFN inducers such as poly(I:C) and LPS [104, 105]. Similar IFN-like activity was seen in snake (*Vipera russelli*) VH2, VSW and lizard (*Gekko gecko*) Gl-1 cell lines infected with the rabies virus [106].

1.5.2 The Expansion and Divergence of the IFN Genes

The type I IFN genes were believed to have originated from a common ancestor and expanded by successive duplications. Figure 1.3 represents a phylogenetic tree of type I IFNs, demonstrating the evolutionary relatedness of the IFN subtypes from different species. Due to the importance of the IFN system in host defense, expansion of the IFN cluster may have occurred initially by selection for redundancy. Once this selection pressure abated they may have diverged and acquired specialized functions or degenerated into pseudogenes [107–109]. Analysis of genomes reveal that newly created genes appear to be governed by positive Darwinian selection, with rapid changes in amino acid sequence and gene structure occurring in very short periods of evolution, particularly within IFN-α subtypes (e.g. feline IFN-α in Fig. 1.3). This positive selection is important in the interaction between structure, function, genotype and phenotype [100, 110]. Some viruses encode proteins such as soluble receptors that are IFN antagonists or other proteins that can disrupt IFN signaling pathways, enabling unhindered viral replication. The presence of multiple IFNs may prevent a viral- or other pathogen-produced IFN antagonist molecule disabling the IFN system completely. Coevolution of the IFN genes and the IFNAR receptors may have also resulted in selection for IFNs with different binding affinities and signaling responses. Thus, the lack of introns may have been another factor in selective pressure towards gene duplication since alternative splicing without introns was not an option to generate multiple functional gene products. In addition to differences in protein-coding sequences, differences between elements in the IFN promoter regions may have evolved a level of control of tissue- or pathogen-specific and temporal regulation of IFN expression, and aid in mounting appropriate host defense responses against different microorganisms. Differences in promoters may also enable IFNs such as trophoblast IFN (IFN-τ) to play physiological roles. Thus, the development of multiple IFN subtypes may account for the diverse range of biological roles these cytokines play.

1.6
Natural and Induced Mutations in IFN Genes

Mutations in type I IFN genes, whether naturally occurring or induced in model organisms and recombinant experimental systems, have provided important insights into the function of these cytokines.

Molecular genetic analysis aimed at linking disease to chromosomal regions identified a highly significant association between serum triglyceride levels and the IFN-α locus on HSA 9p21 in a communal founder population. Alleles at nearby loci are believed to protect against high triglyceride levels, as homozygocity at this locus is associated with low triglyceride levels [111].

Mutations within this gene cluster have revealed the tumor suppressor potential of the IFNs. A number of breakpoints have been identified in the IFN gene cluster, and IFN gene deletions have been associated with gliomas and leukemias, non-small cell lung cancers and others [10, 112–114]. A large number of scaffold/matrix associated regions (S/MARs) flanking functional IFN genes and pseudogenes were identified [115]. S/MARs are involved in shaping the chromatin of DNA into loop domains and in 9p21 these may be involved in organizing the IFN genes into a series of small 2- to 10-kb DNA loop domains which may predispose this region to breaks [115].

Functional genomic methods such as gene knockout studies in mice are invaluable in determining the function of IFNs *in vivo*. Due to the large number of IFN-α genes and their functional redundancies, no *Ifna* gene knockouts have been generated; however, *Ifnar1*-deficient mice have been generated and these are deficient in their responses to all type I IFNs tested, including multiple IFN-α subtypes and IFN-β [116, 117]. IFN-β knockout mice have been produced [118, 119] and are more sensitive to viral infections [119, 120]. When these mice were analyzed, no abnormalities in their CD4 and CD8 T cell populations in peripheral blood, thymus and spleen was observed; however, activated splenic and lymph node T cells showed enhanced proliferation and decreased tumor necrosis factor-α production. A decrease in the number of circulating macrophages and granulocytes was also seen. Tumor growth was aggressive in these knockout mice, demonstrating the potent tumor inhibitory effects of this cytokine. This study demonstrated that IFN-β plays a fundamental nonredundant role in lymphoid development and myelopoiesis [121]. The IFN-β null mice also demonstrated that IFN-β production is necessary for generating an IFN-α response, implying the importance of IFN-β in IFN priming. These mice were also more resistant to septic shock induced by a high dose of Gram-negative bacterial LPS, suggesting the involvement of the type I IFN system in lethality due to septic shock [122]. The IFN-β knockout mice also demonstrated that IFN β plays an important role in the immunoregulation of allergic responses in mice [123] and in the central nervous system as they are more susceptible to chronic demyelinating experimental autoimmune encephalomyelitis [124].

Transgenic mice overexpressing murine IFN-α1 under the control of mouse metallothionein I promoter were generated [125]. In these mice, IFN-α1 was only expressed in the testis, and resulted in degeneration of spermatogenic cells and

atrophy of seminiferous tubules [125]. Surprisingly, these transgenic mice were sterile or turned sterile over time. A similar effect was seen when IFN-β was overexpressed in transgenic mice, where degeneration of spermatids and sterility was observed [126]. These studies suggest that high levels of IFN expression in testis lead to male sterility. When transgenic mice expressing the murine active, hybrid human IFN-α A/D under the control of the human insulin promoter were generated, pancreatic IFN-α expression led to diabetes. Persistent hyperglycaemia with polyuria and polydipsia with inflammation centered on pancreatic islets was observed in these transgenic mice [127]. Neutralizing monoclonal antibodies against IFN-α were able to prevent this inflammation and diabetes [127]. Transgenic mice with targeted overexpression of murine IFN-κ in pancreatic islet β cells resulted in hyperglycaemia [128] and transgenic mice overexpressing IFN-β led to mild hyperglycaemia with 9% developing spontaneous diabetes. These mice were more sensitive to the diabetes-inducing agent streptozotocin, compared to normal mice [129]. These studies suggest that the *IFN* genes may be involved in the pathology of type I diabetes consistent with IFN-α expression in pancreatic islets seen in human patients with diabetes.

Mutagenesis studies of IFN genes have been important in the determination of functionally important domains and residues in the protein. Although all type I IFNs share five helical bundle structures, subtle structural differences may play a role in differential receptor interactions resulting in differential biological potencies or functions. Early studies demonstrated the importance of cysteine residues involved in disulfide bond formation and conserved tyrosine residues for optimal activity [130]. The role of specific amino acid residues in receptor interaction was shown by mutagenesis studies where a double substitution (N86E, Y92D) in the C helix of IFN-β abolished its capacity to induce this receptor association [131]. The ability of IFN-β and not IFN-α to induce the coimmunoprecipitation of both IFNAR-1 and -2 receptors suggested sequence differences between IFNs result in different interactions with the type I IFN receptor complex [132]. The ability of IFN-α and -β to interact with IFNAR-1 and -2 receptors differentially was further elucidated by receptor–ligand mutagenesis studies [133].

1.7 Secondary Structural Features of Type I IFNs

1.7.1 Conserved Amino Acid Residues

Conserved residues are critical for structure and function of IFNs, and analysis of these residues across different species has revealed patterns of evolution of these critical residues. Distinguishing features of mammalian IFNs include five highly conserved proline residues in IFN-αs, but the genes encoding IFN-ω and -τ contain

only four of these conserved prolines, and lack the proline that immediately precedes a conserved cysteine at position 139. The feline IFN sequences all follow the human IFN-α proline pattern and are thus classified as feline IFN-αs [80]. Interestingly, IFN-α subtypes also contain conserved lysine residues near the N-terminus and tyrosine residues near the C-terminus that are critical to optimal activity [131]. Almost all IFN-α subtypes within a species have two conserved disulfide bonds (Cys1–Cys99 and Cys29–Cys139) [134, 135], while IFN-β forms a single intramolecular disulfide bridge equivalent to Cys29–Cys139 in IFN-α. Analysis of position, number and conservation of cysteine residues in different species has assisted in classifying IFN genes found in numerous avian and mammalian (eutherian, marsupial and monotreme) organisms. The cysteine pattern in IFN-α is conserved among mammals with four conserved disulfide bond-forming cysteines present. Only two of the conserved cysteine residues are present in IFN-β. With the exception of murine IFN-β and porcine IFN-δ, most eutherian IFNs conform to this characteristic cysteine pattern.

1.7.2 Post-translational Modifications of Type I IFNs

Glycosylation plays a critical role in protein folding, structure, targeting and pharmacokinetics. Glycosylation is rare among the human IFN-αs, with only two species glycosylated. IFN-α2b is *O*-glycosylated at Thr106 and IFN-α14c is *N*-glycosylated at Asn72 [136, 137]. IFN-α2b is the only IFN-α species with a threonine residue at position 106 and may be the only *O*-glycosylated human IFN-α protein [137, 138]. In virus-infected white blood or lymphoblastoid cells, two differentially glycosylated human IFN-α2 exists as a fully glycosylated form and a form with half the sugar content. In contrast to human IFNs, most murine IFN-α subtypes have a single putative *N*-glycosylation site at Asn78. Human IFN-β has three putative glycosylation sites at positions 29, 69 and 76, while none are present in the murine IFN-β. Interestingly, human IFN-β, unlike the murine protein, is glycosylated at Asn80 at the end of helix C [139]. These sugar residues project away from the core structure and interacts with Asn86 (helix A) and Gln23 (helix C) through hydrogen bond formation. This glycosylation has been shown to reduce the aggregation properties of IFN-β by shielding a hydrophobic patch implicated in oligomerization and is involved in providing temperature stability. When *Escherichia coli*-derived unglycosylated and glycosylated forms of recombinant IFN-β produced in CHO cells were compared, the glycosylated form was 10- to 15-fold more bioactive [139]. Similarly, the glycosylated form of feline IFN-α6 produced in yeast showed considerably higher antiviral activity than the *E. coli*-derived unglycosylated feline IFN-α6 [80]. Thus, glycosylation may contribute to the bioactivity of these proteins.

The two potential *N*-glycosylation sites present at Asn74 and Asn83 in the short BC loop seen in the mature sequence of IFN-ε are similar to those in human IFN-β and -ω, which have a single glycosylation site in this region [13]. The human IFN-ω protein is *N*-glycosylated at a position (Asn78) similar to human IFN-β

[46]. IFN-ζ also contains a single putative *N*-linked glycosylation site at Asn68 [50], while IFN-δ is *N*-glycosylated at Asn79 [140].

1.7.3
Conserved Cysteine Residues and Disulfide Bond Formation

The number and nature of disulfide bonds is critical for maintaining the full biological activity of IFN-α [141]. Curiously, human IFN-β lacks a second disulfide bond present in IFN-α structures, which might explain its different interaction with the receptor [109, 142, 143]. In contrast, murine IFN-β, although structurally similar to its human ortholog, has no intramolecular disulfide bonds [144]. Three cysteine residues are present in human and murine IFN-ε, with the predicted disulfide bond (Cys32–Cys140) linking the top of the AB loop to the top of the E helix. Unlike other IFNs, this protein lacks a disulfide bond connecting the A and C helices. However, secondary structure analysis indicates both human and mouse IFN-ε consist of six and five putative α helices, respectively, whilst the C-terminus helix of human IFN-ε is not present in the mouse protein [13]. Interestingly, IFN-κ in mouse and rat is characterized by the presence of a C-terminal cysteine residue in addition to the four cysteine residues conserved in other type I IFNs [48]. Unusually, IFN-ζ contains four cysteine residues that demonstrate no conservation to those in any other type I IFN [50]. When IFN-ζ was computationally modeled, it formed a structure of five long α helices and a short helix within the BC loop. The Cys52, Cys157 and Cys80, Cys130 residues of IFN-ξ are closer together on the structure and may form disulfide linkages. The unpaired cysteine residues may be involved in intramolecular bonding, resulting in dimer formation. Similarly, the cysteine content in porcine IFN-δ is also unusual, with five cysteine residues at positions Cys9, Cys56, Cys58, Cys107 and Cys145 that are not conserved with those in any other IFN. Four of those are clustered in the same structural region of the molecule, and may form possible disulfide linkages [140].

1.8
The Structure of Type I IFNs

Even though IFNs have only slightly different, closely related amino acid sequences, they display different biological potencies. The bioactivity of these cytokines is determined through specific high-affinity interactions with their cell surface receptors. The availability of large amounts of IFN proteins due to advances in recombinant DNA technology made crystallographic studies feasible in the early 1980s. Due to difficulty in crystallization of these proteins, more than a decade passed before the first IFN crystal structure was determined. The first reported crystals were for human recombinant IFN-α2a [145]. These were of insufficient quality for crystallography and no useful structural information was obtained. Sub-

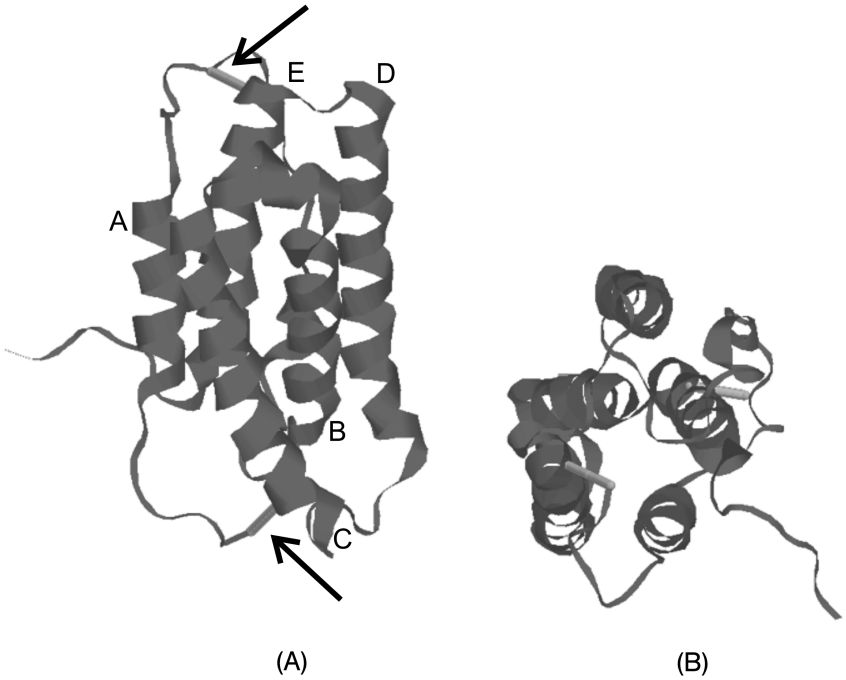

Fig. 1.4. Representative tertiary structure of human IFN-α. Tertiary structure of human IFN-α2 represented as a ribbon diagram, showing the five α-helices connected by loop regions. The disulfide bonds between cysteine residues are indicated by arrows. (A) Side view of the molecule. (B) View from above. (This figure also appears with the color plates.)

sequently, *E. coli*-derived recombinant murine IFN-β crystals were obtained [146, 147], and the crystal structure for murine IFN-β was solved at 2.6 and 2.15 Å resolution, paving the way for better understanding of type I IFN structure [144, 148].

The determination of tertiary structure through nuclear magnetic resonance (NMR) spectroscopy of human IFN-α2a [149], and X-ray crystallographic studies of human IFN-α2b [150], murine and human IFN-β [144, 151], and ovine IFN-τ [152] have determined that the type I IFNs consist of five α helices (labeled A–E) which are linked by a long loop (AB loop) and three shorter loops (BC, CD and DE) (Fig. 1.4). The AB loop contains short segments of 3_{10} helices and encircles helix E with which it is linked by a disulfide bond. In most IFN-α species a second disulfide bond, which is absent in human IFN-β, connects the N-terminus of the molecule to helix C. Even though the core structures of IFNs are similar, large structural differences occur in the AB loops, and at both the C-terminal ends of helix B and BC loops. Interestingly, the crystal lattice of human IFN-β consisted of dimers that contained a zinc atom at the dimer interface. Similar zinc-mediated dimerization of human IFN-α2b was also observed, although murine IFN-β and ovine IFN-τ were crystallized in the monomer form. The physiological relevance

of dimer formation has not been determined. Human and murine IFN-β show only around 50% amino acid sequence identity to the murine form, and the murine protein lacks the conserved disulfide bond-forming cysteine residue pair present in human IFN-β. A 5-amino-acid deletion in the AB loop region is also seen in the murine IFN-β protein. These differences seem to have very little effect on tertiary structure, with the crystal structure of the two proteins being very similar [151]. Human IFN-β displays a tertiary structure typical of type I IFNs, with helix A and B parallel to each other, and antiparallel to C, D and E helices. According to the fold classification of Presnell and Cohen, human IFN-β is classified as a left-handed, type 2 helix bundle that is defined by antiparallel A, B, C and E helices [151, 153].

Mutagenesis studies have shown that the AB loop is critical for high-affinity binding and sequence differences in this region may be important for the difference seen among the IFN subtypes. Hybrid scanning, site-directed mutagenesis and other techniques have identified three functionally important segments on the IFN-β polypeptide sequence. These segments are spatially close to each other, indicating a receptor-binding interface [148]. The NMR structure of IFN-α2 bound to the IFNAR-2 extracellular domain was determined and demonstrated the presence of a predominantly aliphatic hydrophobic patch on the receptor that interacted with a matching hydrophobic surface of IFN-α2 [154]. In addition to this, an adjacent motif of alternating charged side-chains was involved in guiding the two proteins together into a complex [154, 155]. Comparison of the structure of ovine IFN-τ with the human IFN-α2b structure enabled the prediction of binding sites of different IFNs for the IFNAR receptors.

These structural studies, together with mutagenesis and receptor-binding studies, have provided a basis for understanding how structural differences between different subtypes affect biological activity. When the antiviral activities of murine type I IFN subtypes were measured relative to that of IFN-α1, most IFNs had similar antiviral activity (IFN-α2, -α5, -α6/8, -α6T, -α7/10, -α9, -α13 and -α14); however, IFN-α4, -α11, α12, -β and -ζ had up to 8- to 10-fold higher antiviral activity [38, 156–159]. The Arg58 and Asp59 residues have been associated with the high activity of IFN-α4 [38]. Interestingly, the antiproliferative activity of different murine IFN subtypes correlated with antiviral activity, with IFN-α11, -α12, -β and -ζ demonstrating up to 100-fold more potent antiproliferative activity relative to that of IFN-α1 [38]. In addition to differences in their biological potencies, different murine IFN subtypes have been shown to differentially activate STAT signaling [160]. Differences in biological activities and potencies of type I IFNs may therefore result from a combination of factors, such as subtle differences in sequence or structure and differences in receptor interaction or binding affinities [161–164]. Further studies elucidating the structural biology of the ligand–receptor complex will further enhance our understanding of structure–function relationships of IFNs and our ability to manipulate these proteins to improve therapeutic outcomes.

Thus structure–function studies together with evolutionary and phylogenetic analysis of both protein-coding genomic sequences and the regulatory sequences

that dictate their patterns of expression have assisted in elucidating the biology of these therapeutically important cytokines.

References

1 A. Isaacs, J. Lindenmann, *Proc Roy Soc Lond B* **1957**, *147*, 258–267.
2 Y. H. Tan, R. P. Creagan, F. H. Ruddle, *Proc Natl Acad Sci USA* **1974**, *71*, 2251–2255.
3 D. L. Slate, F. H. Ruddle, *Cell* **1979**, *16*, 171–180.
4 C. Chany, C. Finaz, D. Weil, M. Vignal, N. Van Cong, J. de Grouchy, *Ann Genet* **1980**, *23*, 201–207.
5 S. Nagata, N. Mantei, C. Weissmann, *Nature* **1980**, *287*, 401–408.
6 M. Houghton, I. J. Jackson, A. C. Porter, S. M. Doel, G. H. Catlin, C. Barber, N. H. Carey, *Nucleic Acids Res* **1981**, *9*, 247–266.
7 D. Owerbach, W. J. Rutter, T. B. Shows, P. Gray, D. V. Goeddel, R. M. Lawn, *Proc Natl Acad Sci USA* **1981**, *78*, 3123–3127.
8 T. B. Shows, A. Y. Sakaguchi, S. L. Naylor, D. V. Goedell, R. M. Lawn, *Science* **1982**, *218*, 373–374.
9 O. I. O. Olopade, S. K. Bohlander, H. Pomykala, E. Maltepe, E. Van Melle, M. M. Le Beau, M. O. Diaz, *Genomics* **1992**, *14*, 437–443.
10 M. O. Diaz, H. M. Pomykala, S. K. Bohlander, E. Maltepe, K. Malik, B. Brownstein, O. Olopade, *Genomics* **1994**, *22*, 540–552.
11 M. Rubinstein, W. P. Levy, J. A. Moschera, C. Y. Lai, R. D. Hershberg, R. T. Bartlett, S. Pestka, *Arch Biochem Biophys* **1981**, *210*, 307–318.
12 S. J. Humphray, K. Oliver, A. R. Hunt, R. W. Plumb, J. E. Loveland, K. L. Howe, T. D. Andrews, S. Searle, S. E. Hunt, C. E. Scott, M. C. Jones, R. Ainscough, J. P. Almeida, K. D. Ambrose, R. I. Ashwell, A. K. Babbage, S. Babbage, C. L. Bagguley, J. Bailey, R. Banerjee, D. J. Barker, K. F. Barlow, K. Bates, H. Beasley, O. Beasley, C. P. Bird, S. Bray-Allen, A. J. Brown, J. Y. Brown, D. Burford, W. Burrill, J. Burton, C. Carder, N. P. Carter, J. C. Chapman, Y. Chen, G. Clarke, S. Y. Clark, C. M. Clee, S. Clegg, R. E. Collier, N. Corby, M. Crosier, A. T. Cummings, J. Davies, P. Dhami, M. Dunn, I. Dutta, L. W. Dyer, M. E. Earthrowl, L. Faulkner, C. J. Fleming, A. Frankish, J. A. Frankland, L. French, D. G. Fricker, P. Garner, J. Garnett, J. Ghori, J. G. Gilbert, C. Glison, D. V. Grafham, S. Gribble, C. Griffiths, S. Griffiths-Jones, R. Grocock, J. Guy, R. E. Hall, S. Hammond, J. L. Harley, E. S. Harrison, E. A. Hart, P. D. Heath, C. D. Henderson, B. L. Hopkins, P. J. Howard, P. J. Howden, E. Huckle, C. Johnson, D. Johnson, A. A. Joy, M. Kay, S. Keenan, J. K.

Kershaw, A. M. Kimberley, A. King, A. Knights, G. K. Laird, C. Langford, S. Lawlor, D. A. Leongamornlert, M. Leversha, C. Lloyd, D. M. Lloyd, J. Lovell, S. Martin, M. Mashreghi-Mohammadi, L. Matthews, S. McLaren, K. E. McLay, A. McMurray, S. Milne, T. Nickerson, J. Nisbett, G. Nordsiek, A. V. Pearce, A. I. Peck, K. M. Porter, R. Pandian, S. Pelan, B. Phillimore, S. Povey, Y. Ramsey, V. Rand, M. Scharfe, H. K. Sehra, R. Shownkeen, S. K. Sims, C. D. Skuce, M. Smith, C. A. Steward, D. Swarbreck, N. Sycamore, J. Tester, A. Thorpe, A. Tracey, A. Tromans, D. W. Thomas, M. Wall, J. M. Wallis, A. P. West, S. L. Whitehead, D. L. Willey, S. A. Williams, L. Wilming, P. W. Wray, L. Young, J. L. Ashurst, A. Coulson, H. Blocker, R. Durbin, J. E. Sulston, T. Hubbard, M. J. Jackson, D. R. Bentley, S. Beck, J. Rogers, I. Dunham, *Nature* **2004**, *429*, 369–374.

13 M. P. Hardy, C. M. Owczarek, L. S. Jermiin, M. Ejdeback, P. J. Hertzog, *Genomics* **2004**, *84*, 331–345.

14 E. De Maeyer, J. De Maeyer-Guignard, *Int Rev Immunol* **1998**, *17*, 53–73.

15 B. Cederblad, A. E. Gobl, G. V. Alm, *J Interferon Res* **1991**, *11*, 371–377.

16 S. B. Feldman, M. Ferraro, H. M. Zheng, N. Patel, S. Gould-Fogerite, P. Fitzgerald-Bocarsly, *Virology* **1994**, *204*, 1–7.

17 P. Fitzgerald-Bocarsly, *Biotechniques* **2002**, *22 (Suppl 16–20)*, 24–19.

18 S. Pestka, J. A. Langer, K. C. Zoon, C. E. Samuel, *Annu Rev Biochem* **1987**, *56*, 727–777.

19 N. Kadowaki, S. Antonenko, Y. J. Liu, *J Immunol* **2001**, *166*, 2291–2295.

20 P. Kontsek, G. Karayianni-Vasconcelos, E. Kontsekova, *Acta Virol* **2003**, *47*, 201–215.

21 C. D. Krause, S. Pestka, *Pharmacol Ther* **2005**, *106*, 299–346.

22 R. M. Roberts, *Endocine Rev* **1992**, *13*, 432–452.

23 R. M. Roberts, *Nature* **1993**, *362*, 583.

24 F. Lefevre, V. Boulay, *J Biol Chem* **1993**, *268*, 19760–19768.

25 J. M. Trent, S. Olson, R. M. Lawn, *Proc Natl Acad Sci USA* **1982**, *79*, 7809–7813.

26 C. Weissmann, H. Weber, *Prog Nucleic Acid Res Mol Biol* **1986**, *33*, 251–300.

27 Map viewer in NCBI Database. D. A. Benson, I. Karsch-Mizrachi, D. J. Liyman, J. Ostell, D. L. Wheeler, *Nucleic Acid Res* **2005**, *33*, **D34–38.**

28 ENSEMBL genome browser. J. Stalker, B. Gibbins, P. Meidl, J. Smith, W. Syooner, H. R. Hotz, A. V. Cox, *Genome Res* **2004**, *14*, 951–955.

29 J. Xu, S. Gu, S. Wang, J. Dai, C. Ji, Y. Jin, J. Qian, L. Wang, X. Ye, Y. Xie, Y. Mao, *Mol Biol Rep* **2003**, *30*, 239–242.

30 K. Yoshida, *Oncol Rep* **2005**, *13*, 1133–1137.

31 J. A. van der Korput, J. Hilkens, V. Kroezen, E. C. Zwarthoff, J. Trapman, *J Gen Virol* **1985**, *66*, 493–502.

32 V. van Pesch, H. Lanaya, J. C. Renauld, T. Michiels, *J Virol* **2004**, *78*, 8219–8228.

33 A. J. Gentles, S. Karlin, *Trends Genet* **1999**, *15*, 47–49.

34 M. K. Sakharkar, P. Kangueane, D. A. Petrov, A. S. Kolaskar, S. Subbiah, *Bioinformatics* **2002**, *18*, 1266–1267.

35 M. K. Sakharkar, V. T. Chow, I. Chaturvedi, V. S. Mathura, P. Shapshak, P. Kangueane, *Front Biosci* **2004**, *9*, 3262–3267.

36 M. K. Sakharkar, V. T. Chow, P. Kangueane, *In Silico Biol* **2004**, *4*, 0032.

37 M. K. Sakharkar, P. Kangueane, *BMC Bioinformatics* **2004**, *5*, 67.

38 D. N. N. Lee, R. Brissette, M. Chou, M. Hussain, D. S. Gill, M. J. Liao, D. Testa, *J Interferon Cytokine Res* **1995**, *15*, 341–349.

39 M. Ohlsson, J. Feder, L. L. Cavalli-Sforza, A. von Gabain, *Proc Natl Acad Sci USA* **1985**, *82*, 4473–4476.

40 P. J. Hertzog, L. A. O'Neill, J. A. Hamilton, *Trends Immunol* **2003**, *24*, 534–539.

41 R. Hauptmann, P. Swetly, *Nucleic Acids Res* **1985**, *13*, 4739–4749.

42 G. R. Adolf, I. Maurer-Fogy, I. Kalsner, K. Cantell, *J Biol Chem* **1990**, *265*, 9290–9295.

43 D. J. Capon, H. M. Shepard, D. V. Goeddel, *Mol Cell Biol* **1985**, *5*, 768–779.

44 A. Himmler, R. Hauptmann, G. R. Adolf, P. Swetly, *DNA* **1986**, *5*, 345–356.

45 I. Flores, T. M. Mariano, S. Pestka, *J Biol Chem* **1991**, *266*, 19875–19877.

46 P. Kontsek, *Acta Virol* **1994**, *38*, 345–360.

47 G. R. Adolf, *Mult Scler* **1995**, *1 (Suppl 1)*, S44–S47.

48 D. W. LaFleur, B. Nardelli, T. Tsareva, D. Mather, P. Feng, M. Semenuk, K. Taylor, M. Buergin, D. Chinchilla, V. Roshke, G. Chen, S. M. Ruben, P. M. Pitha, T. A. Coleman, P. A. Moore, *J Biol Chem* **2001**, *276*, 39765–39771.

49 B. Nardelli, L. Zaritskaya, M. Semenuk, Y. H. Cho, D. W. LaFleur, D. Shah, S. Ullrich, G. Girolomoni, C. Albanesi, P. A. Moore, *J Immunol* **2002**, *169*, 4822–4830.

50 K. Oritani, K. L. Medina, Y. Tomiyama, J. Ishikawa, Y. Okajima, M. Ogawa, T. Yokota, K. Aoyama, I. Takahashi, P. W. Kincade, Y. Matsuzawa, *Nat Med* **2000**, *6*, 659–666.

51 K. Oritani, P. W. Kincade, Y. Tomiyama, *J Mol Med* **2001**, *79*, 168–174.

52 K. Oritani, Y. Tomiyama, *Int J Hematol* **2004**, *80*, 325–331.

53 K. Oritani, S. Hirota, T. Nakagawa, I. Takahashi, S. Kawamoto, M. Yamada, N. Ishida, T. Kadoya, Y. Tomiyama, P. W. Kincade, Y. Matsuzawa, *Blood* **2003**, *101*, 178–185.

54 S. Kawamoto, K. Oritani, E. Asakura, J. Ishikawa, M. Koyama, K. Miyano, M. Iwamoto, S. Yasuda, H. Nakakubo, F. Hirayama, N. Ishida, H. Ujiie, H. Masaie, Y. Tomiyama, *Exp Hematol* **2004**, *32*, 797–805.

55 R. M. Roberts, T. Ezashi, C. S. Rosenfeld, A. D. Ealy, H. M. Kubisch, *Reprod Suppl* **2003**, *61*, 239–251.

56 M. D. Meyer, P. J. Jansen, W. W. Thatcher, M. Drost, L. Badinga, R. M. Roberts, J. Li, T. L. Ott, F. W. Bazer, *J Dairy Sci* **1995**, *78*, 1921–1931.

57 R. M. Roberts, L. Liu, Q. Guo, D. Leaman, J. Bixby, *J Interferon Cytokine Res* **1998**, *18*, 805–816.

58 K. A. Frazer, L. Pachter, A. Poliakov, E. M. Rubin, I. Dubchak, *Nucleic Acids Res* **2004**, *32*, W273–279.

59 N. Bray, I. Dubchak, L. Pachter, *Genome Res* **2003**, *13*, 97–102.

60 S. Goodbourn, *Semin Cancer Biol* **1990**, *1*, 89–95.

61 T. Maniatis, J. V. Falvo, T. H. Kim, T. K. Kim, C. H. Lin, B. S. Parekh, M. G. Wathelet, *Cold Spring Harb Symp Quant Biol* **1998**, *63*, 609–620.

62 N. Munshi, Y. Yie, M. Merika, K. Senger, S. Lomvardas, T. Agalioti, D. Thanos, *Cold Spring Harb Symp Quant Biol* **1999**, *64*, 149–159.

63 T. Agalioti, S. Lomvardas, B. Parekh, J. Yie, T. Maniatis, D. Thanos, *Cell* **2000**, *103*, 667–678.

64 S. Goodbourn, T. Maniatis, *Proc Natl Acad Sci USA* **1988**, *85*, 1447–1451.

65 C. M. Fan, T. Maniatis, *EMBO J* **1989**, *8*, 101–110.

66 T. Fujita, M. Miyamoto, Y. Kimura, J. Hammer, T. Taniguchi, *Nucleic Acids Res* **1989**, *17*, 3335–3346.

67 T. K. Kim, T. Maniatis, *Mol Cell* **1997**, *1*, 119–129.

68 S. L. Schafer, R. Lin, P. A. Moore, J. Hiscott, P. M. Pitha, *J Biol Chem* **1998**, *273*, 2714–2720.

69 D. Thanos, T. Maniatis, *Cell* **1992**, *71*, 777–789.

70 W. Du, D. Thanos, T. Maniatis, *Cell* **1993**, *74*, 887–898.

71 L. A. Whittemore, T. Maniatis, *Mol Cell Biol* **1990**, *10*, 1329–1337.

72 M. Paste, G. Huez, V. Kruys, *Eur J Biochem* **2003**, *270*, 1590–1597.

73 U. Schultz, B. Kaspers, P. Staeheli, *Dev Comp Immunol* **2004**, *28*, 499–508.

74 C. R. Dearolf, *Cell Mol Life Sci* **1999**, *55*, 1578–1584.

75 T. Miyazaki, N. Nozawa, T. Kobayashi, *Dis Aquat Organ* **2000**, *43*, 15–26.

76 T. Miyazaki, T. Taniguchi, J. Hirayama, N. Nozawa, *Dis Aquat Organ* **2002**, *51*, 135–138.

77 A. L. Hughes, *J Mol Evol* **1995**, *41*, 539–548.

78 A. L. Hughes, R. M. Roberts, *J Interferon Cytokine Res* **2000**, *20*, 737–739.

79 J. D. Thompson, D. G. Higgins, T. J. Gibson, *Nucleic Acids Res* **1994**, *22*, 4673–4680.

80 R. Wonderling, T. Powell, S. Baldwin, T. Morales, S. Snyder, K. Keiser, S. Hunter, E. Best, M. J. McDermott, M. Milhausen, *Vet Immunol Immunopathol* **2002**, *89*, 13–27.

81 N. Nakamura, T. Sudo, S. Matsuda, A. Yanai, *Biosci Biotechnol Biochem* **1992**, *56*, 211–214.

82 G. A. Harrison, L. J. Young, C. M. Watson, K. B. Miska, R. D. Miller, E. M. Deane, *Cytokine* **2003**, *21*, 105–119.

83 G. A. Harrison, K. A. McNicol, E. M. Deane, *Dev Comp Immunol* **2004**, *28*, 927–940.
84 G. A. Harrison, K. A. McNicol, E. M. Deane, *Immunol Cell Biol* **2004**, *82*, 112–118.
85 M. J. Sekellick, A. F. Ferrandino, D. A. Hopkins, P. I. Marcus, *J Interferon Res* **1994**, *14*, 71–79.
86 U. Krempien, I. Redmann, C. Jungwirth, *J Interferon Res* **1985**, *5*, 209–214.
87 M. Kohase, H. Moriya, T. A. Sato, S. Kohno, S. Yamazaki, *J Gen Virol* **1986**, *67*, 215–218.
88 M. J. Sekellick, P. I. Marcus, *Methods Enzymol* **1986**, *119*, 115–125.
89 J. W. Lowenthal, P. Staeheli, U. Schultz, M. J. Sekellick, P. I. Marcus, *J Interferon Cytokine Res* **2001**, *21*, 547–549.
90 C. Sick, U. Schultz, P. Staeheli, *J Biol Chem* **1996**, *271*, 7635–7639.
91 I. Nanda, C. Sick, U. Munster, B. Kaspers, M. Schartl, P. Staeheli, M. Schmid, *Chromosoma* **1998**, *107*, 204–210.
92 J. M. Moehring, W. R. Stinebring, *Nature* **1970**, *226*, 360–361.
93 R. E. Ziegler, W. K. Joklik, *Methods Enzymol* **1981**, *78*, 563–570.
94 M. Suresh, K. Karaca, D. Foster, J. M. Sharma, *J Virol* **1995**, *69*, 8159–8163.
95 C. Sick, U. Schultz, U. Munster, J. Meier, B. Kaspers, P. Staeheli, *J Biol Chem* **1998**, *273*, 9749–9754.
96 R. Nygaard, S. Husgard, A. I. Sommer, J. A. Leong, B. Robertsen, *Fish Shellfish Immunol* **2000**, *10*, 435–450.
97 C. O'Farrell, N. Vaghefi, M. Cantonnet, B. Buteau, P. Boudinot, A. Benmansour, *J Virol* **2002**, *76*, 8040–8049.
98 S. M. Altmann, M. T. Mellon, D. L. Distel, C. H. Kim, *J Virol* **2003**, *77*, 1992–2002.
99 W. H. Yap, A. Tay, S. Brenner, B. Venkatesh, *Immunogenetics* **2003**, *54*, 705–713.
100 M. Long, M. Deutsch, W. Wang, E. Betran, F. G. Brunet, J. Zhang, *Genetica* **2003**, *118*, 171–182.
101 B. Robertsen, V. Bergan, T. Rokenes, R. Larsen, A. Albuquerque, *J Interferon Cytokine Res* **2003**, *23*, 601–612.
102 G. Lutfalla, H. R. Crollius, N. Stange-Thomann, O. Jaillon, K. Mogensen, D. Monneron, *BMC Genomics* **2003**, *4*, 29.
103 S. Hatada, M. Kinoshita, S. Takahashi, R. Nishihara, H. Sakumoto, A. Fukui, M. Noda, M. Asashima, *Gene* **1997**, *203*, 183–188.
104 A. S. Galabov, *Methods Enzymol* **1981**, *78*, 196–208.
105 J. H. Mathews, A. V. Vorndam, *J Gen Virol* **1982**, *61*, 177–186.
106 T. J. Wiktor, H. F. Clark, *Infect Immun* **1972**, *6*, 988–995.
107 M. O. Diaz, *Semin Virol* **1995**, *6*, 143–149.
108 M. O. Diaz, D. Testa, *Biotherapy* **1996**, *8*, 157–162.
109 G. R. Foster, N. B. Finter, *J Viral Hepat* **1998**, *5*, 143–152.
110 D. Liberles, M. Wayne, *Genome Biol* **2002**, *3*, 1018.1011–1018.1014.

111 D. L. Newman, M. Abney, H. Dytch, R. Parry, M. S. McPeek, C. Ober, *Hum Mol Genet* **2003**, *12*, 137–144.

112 K. Ichimura, E. E. Schmidt, N. Yamaguchi, C. D. James, V. P. Collins, *Cancer Res* **1994**, *54*, 3127–3130.

113 G. Sternik, M. G. Pittis, M. Gutierrez, R. A. Diez, L. Sen, *Medicina (B Aires)* **1998**, *58*, 463–468.

114 C. D. James, J. He, E. Carlbom, M. Nordenskjold, W. K. Cavenee, V. P. Collins, *Cancer Res* **1991**, *51*, 1684–1688.

115 P. L. Strissel, H. A. Dann, H. M. Pomykala, M. O. Diaz, J. D. Rowley, O. I. Olopade, *Genomics* **1998**, *47*, 217–229.

116 S. Y. Hwang, P. J. Hertzog, K. A. Holland, S. H. Sumarsono, M. J. Tymms, J. A. Hamilton, G. Whitty, I. Bertoncello, I. Kola, *Proc Natl Acad Sci USA* **1995**, *92*, 11284–11288.

117 U. Muller, U. Steinhoff, L. F. Reis, S. Hemmi, J. Pavlovic, R. M. Zinkernagel, M. Aguet, *Science* **1994**, *264*, 1918–1921.

118 L. Erlandsson, R. Blumenthal, M. L. Eloranta, H. Engel, G. Alm, S. Weiss, T. Leanderson, *Curr Biol* **1998**, *8*, 223–226.

119 R. Deonarain, A. Alcami, M. Alexiou, M. J. Dallman, D. R. Gewert, A. C. Porter, *J Virol* **2000**, *74*, 3404–3409.

120 R. Deonarain, D. Cerullo, K. Fuse, P. P. Liu, E. N. Fish, *Circulation* **2004**, *110*, 3540–3543.

121 R. Deonarain, A. Verma, A. C. Porter, D. R. Gewert, L. C. Platanias, E. N. Fish, *Proc Natl Acad Sci USA* **2003**, *100*, 13453–13458.

122 M. Karaghiosoff, R. Steinborn, P. Kovarik, G. Kriegshauser, M. Baccarini, B. Donabauer, U. Reichart, T. Kolbe, C. Bogdan, T. Leanderson, D. Levy, T. Decker, M. Muller, *Nat Immunol* **2003**, *4*, 471–477.

123 V. Matheu, A. Treschow, V. Navikas, S. Issazadeh-Navikas, *J Allergy Clin Immunol* **2003**, *111*, 550–557.

124 I. Teige, A. Treschow, A. Teige, R. Mattsson, V. Navikas, T. Leanderson, R. Holmdahl, S. Issazadeh-Navikas, *J Immunol* **2003**, *170*, 4776–4784.

125 A. C. Hekman, J. Trapman, A. H. Mulder, J. L. van Gaalen, E. C. Zwarthoff, *J Biol Chem* **1988**, *263*, 12151–12155.

126 Y. Iwakura, M. Asano, Y. Nishimune, Y. Kawade, *EMBO J* **1988**, *7*, 3757–3762.

127 T. A. Stewart, B. Hultgren, X. Huang, S. Pitts-Meek, J. Hully, N. J. MacLachlan, *Science* **1993**, *260*, 1942–1946.

128 G. Vassileva, S. C. Chen, M. Zeng, S. Abbondanzo, K. Jensen, D. Gorman, B. M. Baroudy, Y. Jiang, N. Murgolo, S. A. Lira, *J Immunol* **2003**, *170*, 5748–5755.

129 M. Pelegrin, J. C. Devedjian, C. Costa, J. Visa, G. Solanes, A. Pujol, G. Asins, A. Valera, F. Bosch, *J Biol Chem* **1998**, *273*, 12332–12340.

130 M. W. Beilharz, I. T. Nisbet, M. J. Tymms, P. J. Hertzog, A. W. Linnane, *J Interferon Res* **1986**, *6*, 677–685.

131 L. Runkel, L. Pfeffer, M. Lewerenz, D. Monneron, C. H. Yang, A. Murti, S. Pellegrini, S. Goelz, G. Uze, K. Mogensen, *J Biol Chem* **1998**, *273*, 8003–8008.

132 E. Croze, D. Russell-Harde, T. C. Wagner, H. Pu, L. M. Pfeffer, H. D. Perez, *J Biol Chem* **1996**, *271*, 33165–33168.

133 M. Lewerenz, K. E. Mogensen, G. Uze, *J Mol Biol* **1998**, *282*, 585–599.
134 R. Wetzel, *Nature* **1981**, *289*, 606–607.
135 R. Wetzel, L. J. Perry, D. A. Estell, N. Lin, H. L. Levine, B. Slinker, F. Fields, M. J. Ross, J. Shively, *J Interferon Res* **1981**, *1*, 381–390.
136 T. A. Nyman, N. Kalkkinen, H. Tolo, J. Helin, *Eur J Biochem* **1998**, *253*, 485–493.
137 G. R. Adolf, I. Kalsner, H. Ahorn, I. Maurer-Fogy, K. Cantell, *Biochem J* **1991**, 276 (Pt 2), 511–518.
138 T. A. Nyman, H. Tolo, J. Parkkinen, N. Kalkkinen, *Biochem J* **1998**, *329*, 295–302.
139 L. Runkel, W. Meier, R. B. Pepinsky, M. Karpusas, A. Whitty, K. Kimball, M. Brickelmaier, C. Muldowney, W. Jones, S. E. Goelz, *Pharm Res* **1998**, *15*, 641–649.
140 F. Lefevre, M. Guillomot, S. D'Andrea, S. Battegay, C. La Bonnardiere, *Biochimie* **1998**, *80*, 779–788.
141 M. W. Beilharz, I. T. Nisbet, M. J. Tymms, P. J. Hertzog, A. W. Linnane, *J Interferon Res* **1986**, *6*, 677–685.
142 P. Lamken, M. Gavutis, I. Peters, J. Van der Heyden, G. Uze, J. Piehler, *J Mol Biol* **2005**, *350*, 476–488.
143 L. C. Roisman, D. A. Jaitin, D. P. Baker, G. Schreiber, *J Mol Biol* **2005**, *353*, 271–281.
144 T. Senda, S. Saitoh, Y. Mitsui, *J Mol Biol* **1995**, *253*, 187–207.
145 D. L. Miller, H. F. Kung, S. Pestka, *Science* **1982**, *215*, 689–690.
146 S. Matsuda, G. Kawano, S. Itoh, Y. Mitsui, Y. Iitaka, *J Biol Chem* **1986**, *261*, 16207–16209.
147 S. Matsuda, T. Senda, S. Itoh, G. Kawano, H. Mizuno, Y. Mitsui, *J Biol Chem* **1989**, *264*, 13381–13382.
148 T. Senda, T. Shimazu, S. Matsuda, G. Kawano, H. Shimizu, K. T. Nakamura, Y. Mitsui, *EMBO J* **1992**, *11*, 3193–3201.
149 W. Klaus, B. Gsell, A. M. Labhardt, B. Wipf, H. Senn, *J Mol Biol* **1997**, *274*, 661–675.
150 R. Radhakrishnan, L. J. Walter, A. Hruza, P. Reichert, P. P. Trotta, T. L. Nagabhushan, M. R. Walter, *Structure* **1996**, *4*, 1453–1463.
151 M. Karpusas, M. Nolte, C. B. Benton, W. Meier, W. N. Lipscomb, S. Goelz, *Proc Natl Acad Sci USA* **1997**, *94*, 11813–11818.
152 R. Radhakrishnan, L. J. Walter, P. S. Subramaniam, H. M. Johnson, M. R. Walter, *J Mol Biol* **1999**, *286*, 151–162.
153 S. R. Presnell, F. E. Cohen, *Proc Natl Acad Sci USA* **1989**, *86*, 6592–6596.
154 J. H. Chill, R. Nivasch, R. Levy, S. Albeck, G. Schreiber, J. Anglister, *Biochemistry* **2002**, *41*, 3575–3585.
155 J. H. Chill, S. R. Quadt, R. Levy, G. Schreiber, J. Anglister, *Structure* **2003**, *11*, 791–802.
156 M. Van Heuvel, I. J. Bosveld, P. Klaassen, E. C. Zwarthoff, J. Trapman, *J Interferon Res* **1988**, *8*, 5–14.
157 J. Trapman, M. van Heuvel, P. de Jonge, I. J. Bosveld, P. Klaassen, E. C. Zwarthoff, *J Gen Virol* **1988**, *69*, 67–75.

158 M. Van Heuvel, I. J. Bosveld, A. A. Mooren, J. Trapman, E. C. Zwarthoff, *J Gen Virol* **1986**, *67*, 2215–2222.
159 P. Harle, V. Cull, L. Guo, J. Papin, C. Lawson, D. J. Carr, *Antiviral Res* **2002**, *56*, 39–49.
160 V. S. Cull, P. A. Tilbrook, E. J. Bartlett, N. L. Brekalo, C. M. James, *Blood* **2003**, *101*, 2727–2735.
161 M. Aguet, M. Grobke, P. Dreiding, *Virology* **1984**, *132*, 211–216.
162 A. Meister, G. Uze, K. E. Mogensen, I. Gresser, M. G. Tovey, M. Grutter, F. Meyer, *J Gen Virol* **1986**, *67*, 1633–1643.
163 T. Yamaoka, S. Kojima, S. Ichi, Y. Kashiwazaki, T. Koide, Y. Sokawa, *J Interferon Cytokine Res* **1999**, *19*, 1343–1349.
164 J. Piehler, G. Schreiber, *J Mol Biol* **1999**, *294*, 223–237.