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Chapter 1

Regulation of IFN- γ Expression

John Fenimore and Howard A. Young

Abstract Interferon gamma, referred to here as IFN- γ , is a major component in immunological cell signaling and is a critical regulatory protein for overall immune system function. First discovered in 1965 (Whelock Science 149: (3681)310–311, 1965), IFN- γ is the only Type II interferon identified. Its expression is both positively and negatively controlled by different factors. In this chapter, we will review the transcriptional and post-transcriptional control of IFN- γ expression. In the transcriptional control part, the regular activators and suppressors are summarized, we will also focus on the epigenetic control, such as chromosome access, DNA methylation, and histone acetylation. The more we learn about the control of this regulatory protein will allow us to apply this knowledge in the future to effectively manipulate IFN- γ expression for the treatment of infections, cancer, inflammation, and autoimmune diseases.

Keywords IFN- γ • Transcriptional control • Epigenetic control • LncRNA • MicroRNA • Activator • Suppressor

1.1 Introduction

Interferon gamma, referred to here as IFN- γ , is a major component in immunological cell signaling and is a critical regulatory protein for overall immune system function. First discovered in 1965 [1], IFN- γ is the only Type II interferon identified. Functionally it is a homodimer with an antiparallel interlocking structure, lacking beta sheets but possessing six alpha helices per monomer. In humans the IFN- γ gene is found on chromosome 12q15 [2] and in mice on chromosome 10D2 [3]. The human gene for IFN- γ has four exon regions and three intron regions, covering 4.04 kbps [4]. The receptor complex for IFN- γ , IFNGR1 and IFNGR2, is almost

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1

ubiquitous in all mammalian cells, save erythrocytes. In addition, the structure of IFN- γ is much conserved in studied jawed vertebrates [5].

IFN- γ 's effects on cells are notable, having been shown to modulate the expression of over 2,300 human genes [6]. While of the functions of different interferons are often redundant, a lack of normal IFN- γ expression is linked with cases of heightened weaknesses to some diseases even in the presence of other interferons. As discussed by Kaufmann and Booty et al. [7, 8], when expression of IFN- γ is low, susceptibility to mycobacterial and fungal infections is common; this shows the need for IFN- γ expression to fight disease and depicts the nonredundant functions of IFN- γ 's maintenance of host resistance [7–10].

Unlike other interferons, even though IFN- γ does possess an antiviral capacity, it is more notable for its broader ability to stimulate and modulate the immune system. IFN- γ triggers the immune responses that lead to phagocytosis and increased expression of the MHC receptors on the surface of cells. This is further highlighted by IFN- γ being used to treat chronic granulomatous diseases, a group of inherited immune disorders in which white blood cells fail to control bacterial infections, thus causing severe infections in the skin, liver, lungs, and bone as pathogens are contained in granules, but not consumed via phagocytosis [7]. In turn the presence of high amounts of IFN- γ has been linked to inflammation. In some diseases such as multiple sclerosis, an autoimmune disease involving inflammation in the brain and spinal column, IFN- γ was present in abnormally high levels in affected tissues [12]. IFN- γ has also been found to be involved in other chronic diseases such as Type 1 diabetes, where IFN- γ overexpression has been linked to autoimmune dysfunction, as infiltration by T cells expressing IFN- γ into islets containing pancreatic beta cells results in destruction of beta cells [13, 14].

The activity of IFN- γ is noted as being involved in the activation, growth, and differentiation of T cells, B cells, macrophages, natural killer (NK) cells, and other cell types such as endothelial cells and fibroblasts, thus making it vital to the inflammatory response and to cell-mediated immune responses [4]. The innate and adaptive immune systems rely on controlled IFN- γ expression to preserve the balance between an effective host immune response and the development of autoimmune disease. For this reason, it is very important to understand the pathways that regulate the expression of IFN- γ .

1.2 Transcriptional Controls

IFN- γ gene expression is controlled by a very complex system of regulation. IFN- γ has been noted to be normally expressed by cells from the immune system, such as the natural killer, or NK, cells and natural killer T, or NKT, cells which are both involved in the innate immune response. IFN- γ is also expressed during adaptive immune responses via CD4⁺ Type 1 helper (Th1) T cell, or CD4⁺ cells, and CD8⁺ cytotoxic T lymphocyte effector cells, or CD8⁺ cells. The expression of IFN- γ is one of the defining traits of CD4⁺ Th1 cell-type immune cells [11]. The differentiation

of immune cells into cells that produce IFN- γ or inhibition of such differentiation is also inherent to the amount of IFN- γ that is expressed by a host undergoing an immune response. Production of IFN- γ is controlled at several levels, including epigenetic changes, chromosomal access, cell surface signaling, transcription factor binding to promoters and enhancers, mRNA stability, and long noncoding RNA (lncRNA) interactions with the IFN- γ locus. Given the importance of IFN- γ to host immune function, it is not surprising that this single gene is regulated at many different levels.

Some regions of the genome are noted as being important for the control of IFN- γ expression in specific cell types but not in others. For instance, the region 92–18 bp upstream of the start of transcription is noted as being vital for expression in human T cells and NKT cells, but not NK cells [15]. More work, illuminating the progress of our understanding of IFN- γ epigenetics, has demonstrated the importance of conformational changes to the structure of the IFN- γ locus and factors that affect access to the site [16–18]; this shows there are multiple mechanisms impacting the transcription of this gene.

1.2.1 Epigenetic Control

The role of access to and control of genetic information is an important factor when discussing the process of transcription. The IFN- γ gene is no exception, with several methods of epigenetic control affecting gene expression. However understanding the specific details of this control is a developing science. Specific areas of conserved noncoding sequences, or CNS, are vital for the expression of IFN- γ as relevant CNS regions are present before and after the transcription start site. Of great interest is the difference between their roles in different cell types. For example, it has been reported that CNS-30 can be deleted from NK cells without a loss of IFN- γ expression, while other cells that express IFN- γ require it for full transcription [15]. T cells require the presence of CNS-2, and all NKT and some T cells require the CNS present at +20 [17]. NK cell gene expression may also be enhanced by access to CNS-2 and CNS+20, but these regions are not required for basal IFN- γ gene expression [17]. Alternatively, CNS-16 has different effects depending upon the cell type; in NK cells it upregulates the expression of IFN- γ , but it is not required for transcription, while in the CD4⁺ Th2 cells, suppression of IFN- γ expression requires this region [17].

1.2.1.1 Chromosomal Access/DNA Methylation/Histone Acetylation

Chromatin condensation, by preventing or allowing access to the promoter or other regulatory elements of a gene, is a first step in controlling gene expression. With respect to IFN- γ , expression is regulated first via chromatin modification. This is shown by the differences between nonactivated T cells and activated T cells with

regard to the chromatin density around the IFN- γ gene [17]. The compression of the IFN- γ region has a marked difference when comparing Th1, Th2, and naïve T cells, as observed from a chromatin conformational capture assay [16]. This is also apparent in NK cells, which also have low chromatin density around the transcription start site indicating easier access to the promoter for binding of the required transcription factors and RNA polymerase [17].

Additional regulation of the expression of IFN- γ comes in the form of DNA methylation, which occurs when cytosine is converted to 5-methylcytosine at CpG nucleotides, and as a result prevents direct interactions with specific transcription factors to those regions of the DNA. The IFN- γ CNS-34, IFN- γ CNS-22, the IFN- γ promoter, and IFN- γ CNS+29 are methylated in naïve T helper cells, and methylation is lost as T helper cells progress through their differentiation pathways [19, 20]. The difference in methylation of DNA is notable in cells that do not express IFN- γ , where the locus is highly methylated, indicating that the methylation of specific transcription factor binding sites is an additional mechanism of transcriptional control. Contrasting this, hypomethylation, which is observed between the -200 and +1 positions of the IFN- γ promoter, contributes to the expression of the gene in activated Th1 and NK cells, through binding of transcription factors to those regulatory regions [20]. A target for methylation is a SnaBI restriction enzyme recognition site that is highly conserved in all species and is present at -52 bp in the IFN- γ promoter [17, 18]. Demethylation of this SnaBI site is rapid during the processes of antigen activation, differentiation, and proliferation of T cells [17]. Of importance NK cells do not have notable methylation at this site, and in NK cells, cytokine stimulation of IFN- γ expression (e.g., IL-12) results in the rapid demethylation of the broader IFN- γ locus [17].

Histone acetylation, another method of restricting transcription of a gene, is a reversible modification of DNA that regulates chromatin access. Histone acetylation of the IFN- γ locus is of interest because when naïve T cells are initially stimulated, the loci for the IFN- γ and IL-4 genes are unpacked by histones [17]. The process of histone acetylation changes after differentiation, with 50 kb of DNA both up- and downstream from the IFN- γ locus being closed off in non-IFN- γ expressing CD4⁺ Th2 cells [17]. This can also be observed by depriving the activated Th1 cells of STAT4, thus suggesting that active binding of STAT4 driven by IL-12 is critical for maintaining an open chromatin conformation at the IFN- γ locus [22]. Additionally, in T cells it has been shown that chromatin confirmation is driven through an interplay of T-cell-specific T-box transcription factor (T-bet) and GATA3, which are transcription factors we will discuss later in this chapter. T-bet notably keeps the area where IFN- γ is located accessible via histone acetylation, while GATA3 seems to prevent the action of T-bet in keeping the IFN- γ locus accessible [17, 18]. This interaction between T-bet and GATA3 is notably absent in NK cells as the CNS regions that impact IFN- γ gene expression have, even in the absence of stimulation and active transcription, similar histone-acetylated domains as compared to that found in activated T cells [18].

1.2.1.2 Chromatin Positioning

Chromatin may form blocks to transcription or contrastingly cause specific areas of DNA to be more accessible; this is done not only via compression but also via moving elements away or toward enhancers. Chromatin may also cause movement in the three-dimensional space the gene occupies to prevent the association of transcription factors or to facilitate such binding, via chromatin looping. This accessibility is observed via a notable change in the conformation of the IFN- γ locus [16]. CCCTC-binding factor, or CTCF, is a zinc finger transcription factor that binds the core CCCTC sequence [21]. These proteins have been described as suppressors for which there are three binding sites across the IFN- γ gene locus. These sites have been defined as markers that have been used to denote the limits of this locus [22] and have also been depicted to create a loop in active T cells which has been hypothesized to be required for protection of the gene from heterochromatin silencing and to increase the efficiency of expression [17].

1.2.1.3 LncRNAs

Long noncoding RNAs, or lncRNAs, are noted as being inhibitors of transcription via interactions between sections of RNA greater than 200 bp that are transcribed alongside other regions of chromatin that link to and inhibit the action of proteins and RNA. The role of these lncRNAs in the enhancement or suppression of transcription is a developing field of study as their activity is becoming more defined. For example, the ncRNA repressor of the nuclear factor of activated T cells, or NRON, is a negative regulator of the transcription factor (NFAT), which interacts with the IFN- γ promoter [23]. This inhibition of NFAT by NRON is caused by the formation of protein and RNA complexes, resulting in sequestration of NFAT in the cytosol, away from the DNA [23]. It has also been reported that Theiler's murine encephalitis virus possible gene 1, or TMEVPG1, also named nettoie Theiler's pas Salmonella, or NeST, is the first identified lncRNA to regulate expression of IFN- γ [24]. NeST is located 170 kb downstream from the IFN- γ gene and transcribed from the antisense strand relative to IFN- γ ; the 33 kb NeST gene is spliced into a 1.7 kb RNA transcript in human cells [24]. Studies have shown that NeST's expression in both mouse and human is Th1 T-cell selective and dependent on STAT4 and T-bet [24]. Due to this pattern of expression and its structural traits, it is believed that NeST associates with WD repeat-containing protein 5, or WDR5, to promote histone H3 lysine 4, drive methylation to enhance chromatin accessibility, and conversely decrease attraction of local histones at the nearby IFN- γ locus, thus promoting transcription [17].

1.2.1.4 miRNAs

MicroRNAs, or miRNAs, are between 18 and 25 nucleotides long and are noncoding RNAs that suppress gene expression by binding to the 3' UTR of target genes, resulting in either translation inhibition or mRNA degradation. A broader role for miRNA control of IFN- γ expression was demonstrated by studies demonstrating that the elimination of the Dicer gene, vital for the processing of microRNAs, increased the expression of IFN- γ in T cells [25]. Interestingly this effect has been noted in CD4⁺ TH2 cells indicating that there may be some changes to the epigenetics in the cells as a result of the inhibition of miRNA processing [25].

Recently studies have shown that the miRNA, miR-223, which is notably enhanced by estrogen, positively regulates the expression of IFN- γ via inhibition of anti-inflammatory elements such as IL-10 [26]. In other studies, microRNA-146a and microRNA-146b both suppress the activity of TRAF6 and IRAK1 protein expression, factors that are important for the function of the IFN- γ promoter, and thus cells expressing these miRNAs show decreased IFN- γ expression [27, 28]. Studies have also shown that miR-29 suppress IFN- γ production by directly targeting IFN- γ mRNA and enhancing its degradation [29]. However contrasting experimental evidence reports that miRNA-29 inhibition is driven via degradation of the T-box transcription factor T-bet mRNA instead of the IFN- γ mRNA [30]. Additional studies focused on miR-155 have shown that in NK cells this miRNA increases the expression of IFN- γ when NK cells are stimulated, notably by both IL-18 and IL-12 [31]. miR-155 is observed having its own expression increased when the NK cells are stimulated with IL-18 and IL-12 via downregulating SHIP1, a phosphatase, that in turn suppresses NF- κ B activity, a transcription factor known to bind to the IFN- γ locus and stimulate transcription [31, 32].

1.3 Inducers of IFN- γ

IFN- γ is part of a system of biological signals that can induce substantial changes inside the cell and in the host overall. As such it must be regulated, since it is not passively expressed and different signals regulate expression either by enhancing or inhibiting gene expression. These signals come in many forms, but the primary methods are dependent upon cell activation through signaling via cell surface receptors. Regulation of IFN- γ expression is largely driven by activators like transcription factors such as T-bet and NFAT and specific inhibitors, like TGF- β , in CD4⁺ Type 1 helper T cells. Methods to manipulate this upregulation involve specific systems using phosphorylation of transcription factors, driven by activation of multimeric receptors of cytokines linked to Janus kinases (JAK) activation, while inhibitory signals act through inhibition of these signaling cascades and their receptors resulting in inhibition of the specific transcription factors required for IFN- γ transcription [33–37].

Fig. 1.1 The archetypal motif of the receptor and cytokine structure to stimulate STATs. The interaction of a cytokine causes the dimerization of surface receptors which in turn cause phosphorylation-driven changes which cascade to affect transcription

1.3.1 *IL-2*

IL-2 is a cytokine with three surface receptor subunits, alpha, beta, and a common gamma chain. The presences of these receptor subunits determine the level of affinity in each immune cell type for gene activation. For example, the beta and gamma chains together form a complex that binds IL-2 with intermediate affinity on NK cells, while all three receptor chains form a complex that binds IL-2 with high affinity on activated T cells [33]. The stimulated signaling pathways upregulate IFN- γ expression but also notably upregulate the expression of SOCS1 which functions to prevent overexpression of IFN- γ [38]. Suppressor of cytokine signaling, or SOCS1, is a suppressor of IFN- γ expression that interacts with the tyrosine kinase (TYK), Jak1, and inhibits expression downstream by reducing STAT phosphorylation [33].

IL-2 is also noted for triggering the binding of a STAT5 a/b heterodimer to a STAT5-binding element –3.6 kb from the transcription start site of the IFN- γ gene [39, 40] (Fig. 1.1).

1.3.2 *IL-12/STAT4*

Interleukin 12, or IL-12, is a cytokine heterodimer that can induce IFN- γ transcription and cause nuclear accumulation of IFN- γ mRNA in CD4⁺ T helper cells and NK cells. At activation, two subunits of the IL-12 receptor which is a heterodimer comprised of the beta-1 and the beta-2 chains of the receptor come together to activate the receptor-associated Janus kinases, JAK2 and TYK2 [33, 40]. STAT4 is then phosphorylated by these tyrosine kinases and translocated into the nucleus to activate gene transcription. The pSTAT4 then interacts with IRF-1 and ERM to activate their binding to the IFN- γ promoter [14, 41, 42]. This activation also takes place in NK cells and is independent of most other forms of IFN- γ stimulation [23]. IL-12

has been noted to be dependent on STAT4 to induce expression of IFN- γ as STAT4 is critical for maintaining an open chromatin conformation at the IFN- γ locus [17, 43]. Furthermore transcriptional activity of T-bet, induced by IFN- γ , appears to increase the expression of IL-12 receptor beta 2 [43], thus demonstrating a feedback loop that enhances the cell response to IL-12.

IL-12 stimulation of cells results in an accumulation of IFN- γ mRNA in the nucleus, and release of this accumulated IFN- γ mRNA into the cytoplasm is drastically increased by the presence of interleukin 2, or IL-2 [44]. It has been hypothesized that this increased release of mRNA may be a form of priming cells to release more IFN- γ mRNA when they encounter a second signal, creating a method of regulation that prevents overexpression but allows the cell to have a strong response when needed. It has been shown that both IL-2 and IL-12 in combination promote a steady release of IFN- γ over time, notably more than either signal alone, thus demonstrating a synergistic response of the cell when multiple activating signals are encountered [44].

These previously described pathways of IFN- γ expression when CD4⁺ cells are stimulated by IL-12 can occur only if the T-bet binding region is not blocked by the dual zinc finger transcription factor, GATA-binding protein 3 or GATA3 [45]. The GATA3 transcription factor does not affect the IFN- γ promoter region directly, but instead affects the downregulation of STAT4, thus preventing signaling needed to induce IFN- γ expression. GATA3 is in turn shown to be stimulated by exposure of a naïve T cell to interleukin 4 [45].

STAT3 has been reported to have variable effects in NK cells and their expression of IFN- γ . It has been noted that STAT3 regulates IFN- γ production by binding the IFN- γ promoter upon stimulation of IL-12. However STAT3 has not been shown to be required for IFN- γ expression by NK cells [46, 47].

It is important to note the synergistic effect of cytokines in regard to IFN- γ gene expression. A very strong synergy between IL-12 and IL-18 for IFN- γ expression is driven by the upregulation of receptors on cells that have been exposed to one or the other cytokines; the IL-12 beta-2 receptor chain has been noted to be upregulated by IL-18 and IL-12 can upregulate the IL-18 alpha receptor chain [48, 49], thus stimulating the target cell to be maximally responsive to these cytokines.

1.3.3 IL-15

Interleukin 15, or IL-15, is a heterodimeric cytokine that triggers IFN- γ gene expression through binding to a heterodimeric IL-15 receptor [35]. Signaling occurs through Janus kinases Jak 1 and Jak 3 activation which in turn phosphorylates STAT3 and STAT5. Studies have shown the responsiveness of CD8⁺ cells to IL-15 results in the maintenance of CD8⁺ T-cell survival, thus affecting the host's overall production of IFN- γ [35, 41].

1.3.4 *IL-18*

A member of the IL-1 family, interleukin 18 (IL-18) (originally designated interferon gamma-inducing factor), induces the transcription of IFN- γ . The surface receptors of IL-18 are well characterized; the receptors are heterodimeric with a region for the binding of the ligand, the IL-1 receptor-related protein (IL-1Rrp), and the signal transducing subunit or the accessory protein-like subunit (AcPL) [36]. Once activated, this causes a signal cascade involving MYD88, the IL-1 receptor-associated kinase (IRAK), TNF receptor-associated factor 6, and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) [34]. NF- κ B is shown to bind and enhance activity of the IFN- γ promoter, increasing expression of IFN- γ [50]. NF- κ B additionally undergoes a chain of interactions to stimulate the activation of STAT4 which stimulates the production of IFN- γ [52, 53].

Interleukin 10 (IL-10) is characterized as inhibiting the function of NF- κ B and thus is considered an inhibitor of IFN- γ expression [54].

1.3.5 *IL-27/STAT1/STAT3*

Interleukin 27 (IL-27), a heterodimeric cytokine from the IL-12 family, enhances IFN- γ production by activated T cells and NK cells [33]. One such induction of expression is activated via IL-27's interactions with a dimeric receptor comprised of the interleukin-27 receptor alpha chain (IL27Ra) and glycoprotein 130 (gp130) [55]. Upon binding of IL-27 to this receptor, two arms of Janus kinases are activated: Jak1 linked to the IL27Ra and Jak2, Tyk2, and Jak1 linked to gp130 [55]. Triggering the phosphorylation of STAT1 and STAT3, this causes the transport of the pSTAT1/3 heterodimer to the nucleus where it activates the T-bet promoter, resulting in increased T-bet and subsequent IFN- γ expression [55, 56].

1.4 Positive Transcriptional Control Factors

IFN- γ production is highly variable and cells do not express IFN- γ in a resting state. Thus there are numerous methods to activate immune cells to express this cytokine and vary its levels of expression. These controls are driven by transcription factors that form complexes on specific regions of DNA to control the level of transcription via RNA polymerase 2 [57]. In this section we will discuss the transcription factors involved in regulating IFN- γ expression and briefly summarize our understanding of their activation and activity (Fig. 1.2).

Fig. 1.2 The IFN- γ gene, depicting general structure and relative locations of protein-binding sites

1.4.1 *T-bet*

T-bet, or the T-cell-specific T-box transcription factor, is considered one of the most important controls for the expression of IFN- γ . T-bet has been defined as a key factor in T-cell differentiation and is expressed in many cell types in the immune system. However in many regards, it has been seen as the endpoint that other signal pathways must reach to stimulate the expression of IFN- γ or as the target that needs to be blocked to prevent expression [58]. Recent studies have shown T-bet is required for IFN- γ production and lineage commitment of CD4⁺ T cells, but not of CD8⁺ T cells [45]. Using T-bet KO mice, it was shown that, when stimulated, CD4⁺ T cells had a profound deficiency in IFN- γ expression [59]. Contrastingly it was determined there is little difference in IFN- γ expression in stimulated CD8⁺ cells with or without T-bet [59]. Furthermore IL-12 has also been noted to induce T-bet in an IFN- γ receptor and STAT1-independent manner in CD8⁺ T cells [60]. Induction of T-bet results in upregulation of IL-12RB2, a receptor essential for responsiveness to IL-12, and differentiation to a CD4⁺ Th1 cell type [34]. However studies have shown that T-bet represses the Th2 lineage commitment through a tyrosine kinase-mediated interaction that interferes with the binding of GATA3 to its target DNA [61]. Making T-bet both a suppressor and an enhancer depending on the perspective of Th1/Th2 T-cell determination indicates that T-bet has a broader influence on chromatin structure that directly and indirectly impacts IFN- γ gene expression [17].

1.4.2 *Eomes*

In CD8⁺ T cells, the primary driver of IFN- γ expression is via the transcription factor Eomesodermin (Eomes), which functions independent of T-bet [62]. However it has been reported that there is synergistic effect when both Eomes and T-bet transcription factors are expressed [63]. The activity of Eomes was also noted to increase phosphorylated STAT4 in CD8⁺ T cells, further promoting IFN- γ transcription [64]. Experiments with T-bet^{-/-} CD8⁺ T cells have demonstrated normal IFN- γ expression, while Eomes ^{-/-} CD8⁺ cells did not express IFN- γ at notable levels, indicating a critical role for Eomes in CD8⁺ T-cell IFN- γ transcription [62, 63].

1.4.3 *AP-1/CREB/ATF-2*

Activating promoter 1, or AP-1, has been linked to the activity of the IFN- γ promoter [64]. AP-1 has been reported to enhance the activity of NFAT proteins through the formation of complexes with these transcription factors [42].

It has been shown that c-Jun, cAMP response element-binding protein (CREB), and ATF-2 are essential for activation-induced transcription of IFN- γ , and c-Jun binds preferentially to the IFN- γ proximal element at -52 as a heterodimer with ATF-2 [42]. The c-Fos and c-Jun transcription factors form a heterodimer known as activator protein 1 (AP-1), part of a subset of a large family of transcriptional control proteins, which in turn forms another complex with NFAT [65]. CREB or cAMP response element-binding protein is a known transcription factor closely associated with AP-1 that has been shown to bind to the proximal IFN- γ promoter -73 to -48 bp upstream of the transcription start site [64]. This strongly implicates the involvement of CREB proteins in the regulation of IFN- γ expression [65].

1.4.4 *NFAT*

Nuclear factor of activated T-cell (NFAT) binding sites have been identified at positions -280 bp to -270 bp and -160 bp to -155 bp upstream from the IFN- γ transcription start site, and studies show that they are required for full activity of the IFN- γ promoters in T cells [42, 66]. This effect is documented by experiments that demonstrated decreased levels of expression by full-length IFN- γ promoter-reported constructs with promoter regions that contain mutations in one or both of the NFAT-binding sites [66, 67].

1.5 Negative Transcriptional Controls

Overexpression of a specific signal can lead to drastic problems in all biological systems. In the immune system, such overexpression of a specific gene can cause autoimmune responses that can have far-reaching consequences to a host. IFN- γ is a powerful immunoregulator and the ability to turn the signal off is in many ways just as important as its activation. While not as fully understood as activating signals, characterization of the inhibitory signals leading to decreased expression of IFN- γ is proving vital to our understanding of the regulation of this gene.

1.5.1 *Homeobox/Prox1*

Prospero-related homeobox (Prox1) is a suppressor of the expression of IFN- γ in T cells; however, it does not typically function to suppress the genes as a homeobox gene would normally be expected to function. It has been reported that Prox1 does not bind to the promoter region of IFN- γ but that it uses a method of facilitating a conformational change with a protein intermediary to inhibit the synthesis of IFN- γ mRNA [68]. The nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR γ) has been shown to link to the Prox1 structure and inhibit IFN- γ expression [68]. The presence of Prox1 however is also notably downregulated as the cell differentiates into a CD4⁺ T cell, but the mechanism is unknown [68]. PPAR γ and PPAR delta have also been reported to inhibit IFN- γ gene expression in part by antagonizing the activities of the transcription factors AP-1, STAT, and NF- κ B [69, 70]. However, once IFN- γ has been expressed, it has been demonstrated to in turn suppress PPAR γ expression via upregulation of activated STAT1 [71], thus forming a regulatory cycle to prevent under- or overexpression.

IFN- γ has been linked to many sex-biased autoimmune diseases and the sex bias in PPAR γ coincides with these phenomena [72]. Males have a notably higher level of expression of PPAR γ than females, thus contributing to the finding that female mice and women express more IFN- γ than their male counterparts [72].

1.5.2 *TGF-B*

Transforming growth factor beta (TGF-B) is a superfamily of cytokines that, when activated, downregulate the expression of T-bet mRNA via direct interaction of SMAD proteins with the T-bet promoter [73]. TGF- β utilizes SMAD2, SMAD3, and SMAD4 to suppress IFN- γ via inhibiting the activity of T-bet by binding to the proximal T-bet site in the IFN- γ locus [74].

1.5.3 DREAM

Downstream regulatory element antagonist modulator (DREAM) can directly bind to the IFN- γ promoter and inhibit transcription. DREAM forms a complex with the related protein KChIP-2 around an area downstream of the TATA box in the IFN- γ promoter [75]. DREAM's binding takes place as a doublet with one direct and one inverted downstream regulatory element located at position +20 downstream from the transcription initiation site if IFN- γ , thus blocking transcription [75]. DREAM is constitutively expressed in unstimulated T lymphocytes, and it has been reported that DREAM is rapidly downregulated after stimulation of T cells [75]. This suggests DREAM may be important to prevent basal expression of IFN- γ when immune cells are not stimulated and are in a resting state.

1.5.4 GATA3

Transacting T-cell-specific transcription factor GATA3 is a potent suppressor of IFN- γ and has effects on T-cell differentiation. Members of the CREB/ATF, AP-1, octamer 1, and GATA families of transcription factors bind to the proximal -70 bp to -47 bp and distal -98 bp to -72 bp regions upstream from the start site of IFN- γ transcription [76]. It has been reported that GATA3 restricts access to the promoter regions of IFN- γ and T-bet, thus preventing interactions of activating transcription factors with the IFN- γ promoter [76]. In addition, Gata3 and octamer 1 enhance the expression of IL-4-driving cell differentiation away from a state where IFN- γ is expressed [77].

Published reports have promoted the idea that even though the binding of GATA3 can be detected at two GATA motifs, in positions -108 bp to -91-bp, of the human IFN- γ gene, it may be that the lack of expression of IFN- γ in the presence of GATA3 is more notably driven by the suppression of other transcription factors [76, 78]. Of note, there was a reduction in STAT4, involved in promoting IFN- γ transcription, in the presence of high levels of GATA3 [76]. Interestingly, these studies also showed a reduction of the amount of STAT1 in the cells expressing elevated levels of GATA3 [78]. In addition, runt-related transcription factor 3 (Runx3) enhances the binding of T-bet to the IFN- γ locus by binding to specific regions in the IFN- γ promoter; this in turn decreases the binding frequency of GATA3 [79]. The binding of T-bet acts to competitively suppress GATA3 binding, thus enhancing IFN- γ transcription [79, 80]. GATA3 has also been linked to changes in histone modulation, promoting access to some areas and restricting access to others in competition with T-bet [76].

1.5.5 *Yin Yang 1*

Ying Yang 1, or YY1, is a zinc finger transcription factor that belongs to the human GLI-Kruppel family of nuclear proteins and is an inhibitor of the IFN- γ promoter in two notable models [81]. There are two constitutive YY1-binding sites in the IFN- γ promoter at positions Y1 –199 bp to –203 bp and Y2 –217 bp to –221 bp upstream from the transcriptional initiation site [81]. These sites are both hypothesized to play a role in inhibiting the expression of IFN- γ by competition for DNA binding with AP-1 at the Y1 position [64] and by activation of an AP-2-like protein by YY1 binding at the Y2 position which then acts as a repressor of IFN- γ transcription [81].

1.6 Posttranscriptional Regulation

Posttranscriptional downregulation of IFN- γ is mostly characterized as being from processes degrading the IFN- γ mRNA. Removing the region in the 3' UTR that contains the AUUA repeat elements (ARE) results in significantly higher levels of expression of IFN- γ due to stabilization of the mRNA [82]. A second method of control involves the 5' UTR region of the mRNA. As the structure of the 5'UTR has revealed the presence of a semi-knot, this structure triggers a natural anti-dsRNA reaction by RNA-activated protein kinase (PKR) which helps downregulate the continued expression of IFN- γ via binding to the mRNA and facilitating degradation of the dsRNA present in the semi-knot [83, 84].

There are other forms of posttranscriptional controls for IFN- γ . A mechanism by which IFN- γ is posttranslationally modified, i.e., glycosylation at positions 25 and 97, stabilizes the protein and protects it from degradation [85]. In response to stress, the host cell can also change the protein's final structure, by downregulating the expression of the enzyme, Furin, which is needed to generate the IFN- γ protein into a functional state by cleaving the leader peptide [86]. This demonstrates that controlling functional expression of this cytokine is a continuum of mechanisms that extend beyond the domain of transcription alone.

1.7 Conclusions

Negative and positive controls of IFN- γ expression must remain in balance to prevent the promotion of autoimmune diseases or host deficiencies in fighting disease. The conserved nature and the wide-ranging effects of IFN- γ on the adaptive and innate immune systems ensure that the expression of IFN- γ is a potent response required to fight disease, but one that must be controlled in healthy hosts. This control is transcriptionally regulated at the level of chromatin access involving chromatin secondary and tertiary structure, DNA methylation, and histone acetylation. This process is in turn effecting and affected by transcription factors, which are both

activators and inhibitors of gene expression. The specific host factors can be shaped by the process of transcription itself, as impacted by extracellular signals received by the target cells and RNA stability following gene induction. Understanding the labyrinthine system of controls may seem daunting, and the complete picture of IFN- γ control is still a point of active investigation. However, the more we learn about the control of this regulatory protein will allow us to apply this knowledge in the future to effectively manipulate IFN- γ expression for the treatment of infections, cancer, inflammation, and autoimmune diseases.

(The author would like to apologize for any articles or work that was missed in the collection of this work. The volume of data, space, and time constraints made it impossible for the author to create a complete list of the valuable research that has been done on this integral part of immunological research.)

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