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IFNA2: The prototypic human alpha interferon

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Abstract

The human interferon α2 (IFNα2) was the first highly active IFN subtype to be cloned in the early eighties. It was also the first IFN and the first cytokine to be produced and commercialized by the pharmaceutical industry. *Ipso facto* it became the favourite IFNα subtype for academic researchers. For this fortunate reason IFNα2 has been at the origin of most discoveries related to the mechanism of action of type I interferons.

Keywords

Cytokine; type I interferon; interferon α2

1- Introduction

Type I interferons (IFNs) were discovered in 1957 by Isaacs and Lindenman who reported that cells infected with an inactivated virus release a soluble factor exerting an antiviral action (Isaacs and Lindenmann, 1957). We now know that IFN is a key cytokine of the innate immune response which is produced upon recognition of many pathogens and damage-associated molecular patterns released by infected cells or dying cells (Tomasello et al., 2014).

Type I IFNs are capable to act on virtually all body cells since they recognize a receptor which is ubiquitously expressed. In addition to their function in establishing an antiviral state, they are also capable to decrease the proliferation rate of dividing cells and to exert immunomodulatory activities. Type I IFN action impacts not only innate immunity, but also on almost every aspect of cellular and humoral adaptive immune responses. In particular, the action of IFN on dendritic cells (DC) is crucial. It induces IL-15 trans-presentation for NK cells activation. More importandly, IFN can modulate all 3 types of signals delivered by DC to T cells: MHC-antigenic peptide complex, costimulation and cytokine production (Tomasello et al., 2014; Rizza et al., 2015). Type I IFNs are thus critical cytokines for the

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generation of a protective immune response. When given exogenously as a drug to adult mice, IFN exert a potent antitumor effect (Gresser, 2007). In human, IFNα2 has the longest record of clinical use for the treatment of many types of cancer, including some hematological malignancies and solid tumors (Antonelli et al., 2015). In the last 3 years, it has been demonstrated that type I IFNs are essential in processes of immunosurveillance, tumor rejection and regulation of metastasis spread (Diamond et al., 2011; Fuertes et al., 2011; Bidwell et al., 2012). Moreover, IFNs were shown to improve the efficacy of classical antitumor treatments such as radiotherapy, chemotherapy and monoclonal antibodies-based therapy (Burnette et al., 2011; Schiavoni et al., 2011; Stagg et al., 2011).

The type I IFNs represent a family of several closely related subtypes. At least 8 subclasses: α, β, δ, ε, κ, τ, ω and limitin have been described in different mammalian species. They all act through the same plasma membrane receptor made of IFNAR1 and IFNAR2. Humans have 17 subtypes: 13α, 1β, 1ω, 1ε and 1κ (Pestka et al., 2004).

Genes and cDNAs encoding human type I IFNs were first cloned in the early eighties (Derynck et al., 1980; Goeddel et al., 1980; Nagata et al., 1980b; Taniguchi et al., 1980). Among them, the IFNα1 and α2 were the first two α subtypes characterized. Given the low specific activity exhibited by IFNα1, IFNα2 was the first highly active human IFN made available to scientists and physicians. IFNα2 thus became the prototypic type I IFN subtype used in fundamental research and most clinical applications. With some rare exceptions, the basic knowledge generated by these studies is essentially valid for the other α's, β and ω IFN subtypes in humans and higher mammals. In this review, we will summarize what we know on the structure, mechanism of action and biological activities of IFNα2. When pertinent, we will emphasize its uniqueness with respect to the other family subtypes.

2- IFNα2 gene and expression

Soon after the cloning of the human IFNα2 cDNA (Goeddel et al., 1980; Streuli et al., 1980), the gene was isolated and mapping of the type I IFN gene family began (Nagata et al., 1980a; Lawn et al., 1981). All type I IFN genes are clustered on a region covering 400 kb on the short arm of chromosome 9 (Diaz et al., 1994). All are intronless genes, suggesting that the family has originated from a retroposition event replacing, in higher vertebrates, ancestral intron-containing IFN genes (Qi et al., 2010). If all eutherian mammals have several type I IFN genes, the diversification of the family seems to have arisen independently in each species. Thus, except for very closely related species (eg. human and chimpanzee), there is no orthologues of human IFNα2 in other species (Woelk et al., 2007).

Several IFNα2 alleles have been described. The best known are α2a and α2b, both commercialized for clinical use as RoferonA and IntronA, respectively. They differ by a neutral K/R substitution at position 23 (von Gabain et al., 1990). In human populations, the gene encoding IFNα2 is found to be under constraints which prevent mutations, suggesting an essential role in physiology (Manry et al., 2011).

The expression of type I IFN genes is regulated primarily at the transcriptional level. Upstream of the transcription start site and the TATA box, the IFNα2 promoter contains

several virus-responsive elements (VREs), also called positive regulatory domain-like elements (PRD-LEs) (Honda et al., 2005; Genin et al., 2009). These elements are found in the promoter of all IFNα genes. Of relevance, the IFNβ promoter contains two additional regulatory domains engaging NF-κB and ATF-2/c-Jun transcription factors. The VREs are activated by two IFN regulatory factors (IRF) primarily responsible for the initiation of IFNα transcription, IRF-3 and IRF-7. The small variations in the VRE sequences of the different IFNα genes affect the affinities of IRF-3 and IRF-7 binding and may account for some temporal and quantitative differences in gene expression (Genin et al., 2009). While IRF-3 is constitutively expressed in almost all cells, IRF-7 is constitutively expressed at high level mainly in plasmacytoïd dendritic cells (pDCs). IRF-7 is however robustly induced in all cells by type I IFNs. In most cell types, a full IFNα gene expression is thus dependent on a positive feedback loop where the *de novo* synthesis of IRF-7 is critical (Honda et al., 2005).

To act as transcription factors IRF-3 and IRF-7 need to be serine phosphorylated, to homo- or hetero-dimerize and translocate to the nucleus (Fig. 1). Several kinases such as Tank-binding kinase 1 (TBK1) or inducible IκB kinase (IKKε) can mediate phosphorylation. These IRF kinases are themselves activated through signalling cascades initiated by the recognition of many molecular patterns signing virus replication, bacterial infections, viral nucleotide sequences or cellular stresses (Tomasello et al., 2014). If all cells are equipped with the necessary sensors of viral replication, only specific cell types can detect danger patterns upon endocytosis of infected material. Among them pDCs selectively express Toll-like receptor (TLR) 7 and 9 which recognize endosomal ssRNA and DNA with unmethylated CpG motifs, respectively. Since pDCs constitutively express IRF7 and are equipped with a robust protein synthesis and secretion system, they are able to produce very high levels of all IFNα subtypes (Coccia et al., 2004; Ito et al., 2006).

3- IFNα2 structure, receptor binding and signalling

The IFNα2 gene encodes a 188 amino acids precursor that contains a N-terminal 23 amino acids leader sequence that is cleaved during the secretion process. The size of the mature IFNα2 is thus 165 amino acids, which is one amino acid shorter than all other human IFNα subtypes. From sequence alignment, it is clear that the aspartic acid at position 44 in all human IFNα subtypes is missing in IFNα2. No functional property was associated with this deletion. Because IFNα2 is not glycosylated, the biopharmaceutics industry has chosen to produce IFNα2 in *Escherichia coli* fermenter. Up to now, IFNα2 has been classically purified from inclusion bodies and refolded but new methods achieving high soluble protein expression and fast purification are being developed (Bis et al., 2014). The three-dimensional structure of IFNα2 was revealed by nuclear magnetic resonance (NMR) spectroscopy (Klaus et al., 1997) and X-ray crystallography (Radhakrishnan et al., 1996). It contains five α-helices (A to E), the helices A, B, C and E forming a left-handed four helix-bundle characteristic of the helical cytokine family. The long loop between helix A and helix B is perpendicular to the bundle axis; it is linked to helix E by a disulfide bond between cysteine 29 and 138. A second disulfide bond connects the amino-terminal cysteine to position 98 in the helix C.

Type I IFNs exert their biological activity by assembling a ternary IFN-receptor complex with the IFNAR1 and IFNAR2 chains. The structure of the ternary complex IFNAR1-IFNα2-IFNAR2 has been recently solved by X-ray crystallography (Thomas et al., 2011). An excellent recent review analyses in-depth all structural and dynamic aspects of IFNα2 binding (Piehler et al., 2012) and the cartography of the IFNα2 residues interacting with IFNAR1 and IFNAR2 are easily found at Protopedia (http://www.proteopedia.org/wiki/index.php/Journal:Cell:1). Briefly, a surface area of 18 nm^2 formed by part of the helices A and E, and the A–B loop interacts with IFNAR2. The domain of interaction with IFNAR1 is located on the opposite side of the IFN molecule and covers a surface area of 22 nm^2, containing residues located in the helices B, C and D. It is highly probable that all type I IFN subtypes, α, β and ω, form a similar ternary complex as suggested by the perfect superimposition of two ternary complexes assembled by two different IFNs described by Thomas et al. (Thomas et al., 2011), and by other low and high resolution structural data (Chill et al., 2003; Quadt-Akabayov et al., 2006; Li et al., 2008; Strunk et al., 2008; de Weerd et al., 2013).

All IFN subtypes show a dissymmetry in their affinities for the individual IFNAR chains. Typically, the constant of dissociation (K_d) of the interaction with IFNAR1 is in the μM range and the K_d for IFNAR2 is much lower, being in the nM range (Piehler et al., 2012). Since IFNAR1 and IFNAR2 are not pre-assembled at the cell surface (Wilmes et al., 2015), it is likely that IFN binds first to IFNAR2 and then to IFNAR1 in a bi-dimensional reaction to form a ternary complex. Only the ternary complex is capable of signal transduction through the activation of the JAK kinases associated with the receptor chains, the ensuing phosphorylation of STAT1 and STAT2 and the formation of the ISGF3 transcription factor which ultimately induces the transcription of the large family of IFN-induced genes (Borden et al., 2007) (Fig. 2). In addition to this main JAK/Stat signalling pathway initiated by IFN in all cell types, other signalling factors can be activated in a cell type-dependent manner. These include other STAT proteins such as STAT3, STAT4 and STAT5, and elements of p38 and ERK MAPK pathways, PI3K signalling and PKC isoforms (Platanias, 2005). *In fine*, the expression of over 1000 genes can potentially be induced by IFN. It was suggested that such a high number of induced genes (ISG) is necessary to ensure a large spectrum of antiviral action, the replication of each virus being targeted by a unique set of antiviral proteins (Schoggins and Rice, 2011). As a consequence, IFN exhibits a high level of pleiotropic action and can deeply affect the homeostasis of several biological systems. Among these IFN-induced genes, several encode proteins needed for signalling attenuation (Coccia et al., 2006). The most specific and critical negative feed-back regulator of the JAK/STAT pathway activated by IFN is USP18 (Malakhova et al., 2006). This protein binds to the receptor and decreases the stability of the IFN-receptor ternary complex. Moreover, cells where USP18 accumulates become more refractory to IFNα2 than IFNβ, indicating that USP18 is an important determinant of IFN subtype differential activity (Francois-Newton et al., 2011; Wilmes et al., 2015).

The specific activity of a given IFN for a given biological response is function of the stability of the IFN-receptor ternary complex, stability being determined by the individual affinity to IFNAR1 and IFNAR2 (Kalie et al., 2008). The slope of the linear affinity-activity correlation is not necessary the same for all biological activities. For example, on WISH

cells, the slope of the antiproliferative specific activity versus affinity is higher than the slope establishing the relationship between the anti-VSV specific activity and the global affinity of the IFN for IFNAR1 and IFNAR2. This explains why the different type I IFN subtypes exhibit differential activities (Kalie et al., 2008; Levin et al., 2011; Piehler et al., 2012). The binding and activity of all human IFNα subtypes has been compared in a recent study (Lavoie et al., 2011). Except IFNα1 that binds weakly, all other IFNα, including IFNα2, have a similar range of affinity and activity, all weaker than those of IFNβ.

4- IFNα2 biological functions

Stricto sensu, nothing is known about functions unique to IFNα2 in human physiology. Probably its function is integrated with that of all other IFNα since, contrary to IFNβ, there is no known context where IFNα2 is specifically produced as an isolated subtype. Indeed, if IFNα2 is the main IFN subtype that has been used for most *in vitro* experiments to elucidate IFN signalling and mechanism of action, almost all physiological and functional data of type I IFN have been generated in mice, notably through the study of knock-in and knock-out animal models generated in the last decade.

Many aspects of the cellular functions that are modulated by type I IFNs. have been reviewed recently (Trinchieri, 2010; Gough et al., 2012; Tomasello et al., 2014; Crouse et al., 2015; Gajewski and Corrales, 2015; McNab et al., 2015). The important point that emerged is that type I IFN, probably only IFNβ, must be produced at low level under steady-state conditions to ensure the ontogeny and function of several cell types. In the absence of such constitutive production, the homeostasis of the hematopoietic system is perturbed and mice are more susceptible to infection, cancer and have increased bone degradation. Following viral infections or pathogen and stress-associated molecular patterns recognition, IFNα are transiently produced and become detectable in peripheral blood. This IFN not only will prevent viral spread but also will critically orchestrate the set-up of an immune adaptive response. Many pathogenic viruses have developed mechanisms to escape IFN action by inhibiting its production and/or its action (Haller and Weber, 2007; Misasi and Sullivan, 2014). On the other hand, IFN can have detrimental effects in bacterial, parasitic and fungal infections (Trinchieri, 2010; Tomasello et al., 2014; McNab et al., 2015; Stifter and Feng, 2015). These observations explain the well known risk of secondary bacterial infection upon a primary viral attack. Although the mechanisms are complex, and often differ depending of the secondary infection, the general concept is that the IFN produced in response to the primary viral infection inappropriately polarizes the immune responses, compromising the recruitment of IFNγ-dependent anti-microbial innate effector cells.

A balanced IFN production and a balanced IFN response are mandatory to avoid deleterious effects. Subtle dysregulations in the control of these pathways can be disruptive to the equilibrium of the immune system (Tomasello et al., 2014). Systemic lupus erythematous and Sjogren's syndrome are two examples of autoimmune/inflammatory diseases caused and/or worsened by unbridled IFN responses. Compelling evidence of the harmful effect of excess IFNα has come in recent years from the identification of rare monogenic Mendelian disorders which have been grouped as interferonopathies. Patients with the rare Aicardi-Goutières syndrome (AGS) have been extensively studied and causal mutations identified.

AGS patients present with severe auto-inflammation affecting mostly the brain and the skin, though a broad spectrum of other clinical phenotypes have been found (Crow, 2015). The genetic basis of AGS has been defined in a number of families. These disorders are genetically different (caused by mutations in different genes), but are all associated with a disturbance in IFNα homeostasis. Rare patients with an impaired response to type I IFN have been reported (Bogunovic et al., 2012; Zhang et al., 2015). In these individuals the complete deficiency of ISG15, a ubiquitin-like modifier, is responsible of high susceptibility to the Bacillus Calmette-Guérin strain used in vaccination against tuberculosis. Moreover, ISG15 deficiency leads to sustained signaling to type I IFNs, autoantibodies production and the development of inflammation targeting the brain. Hence this autoimmune disorder shares similarities with AGS. Importantly, a deregulation of the IFNα system - whether excess production or response - gives rise to a high baseline level of *ISG* transcripts (a type I IFN signature) in blood cells. Thus, measuring such signature may become a routine screening test in the clinic to identify new interferopathies. Moreover, the recognition of a diverse spectrum of autoimmune phenotypes with excess IFNα calls for strategies of rational intervention with anti-IFN, anti-IFN receptor antibodies or inhibitors of Janus kinases.

5- IFNα2 in the clinic

Except in intra-lesional application, IFNα2 is administered parenterally in order to avoid proteolysis. Among IFNα formulations available in clinical practice, recombinant IFNα2 and pegylated forms (PEG-IFNα2) are the most widely used as antiviral and antitumoral agents. Polyethylene glycol (PEG) covalently linked to IFNα2 improves its pharmacokinetic properties, allowing single weekly administration instead of several administrations per week with standard IFNα2.

Since its first approval for the treatment of hairy cell leukemia in 1986, recombinant IFNα2 has been approved for the treatment of chronic viral hepatitis B (HBV), chronic viral hepatitis C (HCV), chronic myeloid leukemia (CML), Kaposi sarcoma, follicular lymphoma, renal cell carcinoma (RCC), melanoma, T cell lymphoma, multiple myeloma and condylomata acuminate (Antonelli et al., 2015). IFNα2 was also the first available therapy for the treatment of chronic HCV (Hoofnagle et al., 1986). The association of ribavirin and IFNα2 significantly increased the rate of sustained virological response (SVR) (McHutchison et al., 1998). The availability of PEG-IFNα2, more efficient in HCV than IFNα2 (Zeuzem et al., 2000), further enhanced the SVR rate when associated with ribavirin (Manns et al., 2001). PEG-IFNα2/ribavirin was thus the standard of care of chronic HCV.

Antiviral agents against HBV, and more recently against HCV, have challenged the use of IFNα2. Directly acting antiviral agents (DAA), including protease and polymerase inhibitors interfering with HCV replication, are now available. These DAA are well-tolerated drugs that induce a high rate of SVR in a shorter period of treatment. Thus, DAA, initially administered in combination with PEG-IFNα2/ribavirin, are currently investigated in IFN-free regimens (Cortez and Kottilil, 2015). Similarly, targeted therapies are progressively replacing IFN-based therapy of metastatic RCC (VEGF and mTOR inhibitors) and melanoma (BRAF inhibitors). For advanced follicular lymphoma, rituximab is approved in the induction phase (associated with chemotherapy) and in the maintenance setting.

Similarly, drugs such as thalidomide, lenalidomide, bortezomib have replaced IFNα2 in the treatment of multiple myeloma (Antonelli et al., 2015).

IFNα2 has re-emerged in the treatment of patients suffering from CML (chronic myelogenous leukemia). IFNα2 was the first drug to significantly improve CML patients' survival compared to chemotherapy (Talpaz et al., 1987). Since the availability of tyrosine kinase inhibitors (TKI), IFNα2 was no longer considered, until clinical data suggested that IFNα2 pre-treated patients experienced delayed relapses after TKI discontinuation (Rousselot et al., 2007). It was also reported that the association of IFNα2 to TKI significantly improves the major molecular response rate (Preudhomme et al., 2010). Several mechanisms could explain the potential benefit of combining IFNα2 to TKI (Talpaz et al., 2013). The combo therapy is thus currently investigated in clinical trials since TKI do not appear to be curative in CML. Yet, the major limitation of IFNα2 use in CML is its adverse effects which limit its full clinical benefit (Guilhot et al., 2009).

In general, the major limiting factor in the clinical use of IFNα2 is the occurrence of induced side effects that alter the patients' quality of life and often require dose reduction or premature interruption (Sleijfer et al., 2005). When monitoring patients receiving IFNα2, the main concern is neurological toxicity, with depression and suicidal ideation, symptoms that should be detected and carefully managed. Flu-like syndrome, characterized by fever, chills, myalgia, and headache, almost always occurs but is rarely dose-limiting contrary to the other adverse effects. Among these latter, hematotoxicity results from different mechanisms including inhibition of lymphocyte egress from lymph nodes (Shiow et al., 2006) and inhibition of platelet production (Yamane et al., 2008). Chronic side effects include asthenia, weight loss and autoimmune complications such as thyroid dysfunction, diabetes and dermatological manifestations. Other side effects (hepatic, renal, pulmonary, cardiovascular, gastro-intestinal) are less frequent but can be life-threatening.

A correlation has been found between clinical efficacy of IFNα2 in patients treated for melanoma and the appearance of autoimmune manifestations (Gogas et al., 2006). Thus, side effects can greatly limit treatment efficacy and are likely related to the pleiotropic activity of IFN and its ability to act on all cells in the body. In this regard, methodologies to increase the cellular specificity of IFN action are being developed (Garcin et al., 2014; Tomasello et al., 2014; Uze and Tavernier, 2015). These targeting strategies may provide new IFN formulations, more efficient, better tolerated, and could lead to a revival of this old drug with untapped potential. However, the cell types on which IFN has to act in order to establish the wanted physiological effects are far from being identified. Their characterization is undoubtedly a challenge to the ongoing academic research.

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Highlights

- IFNA2 was one of the first type I interferon gene cloned in the 1980's
- IFNα2 is one of the most potent interferon α
- IFNα2 is the interferon α subtype used in the clinic
- Its gene structure, expression, signalling and biological functions are reviewed

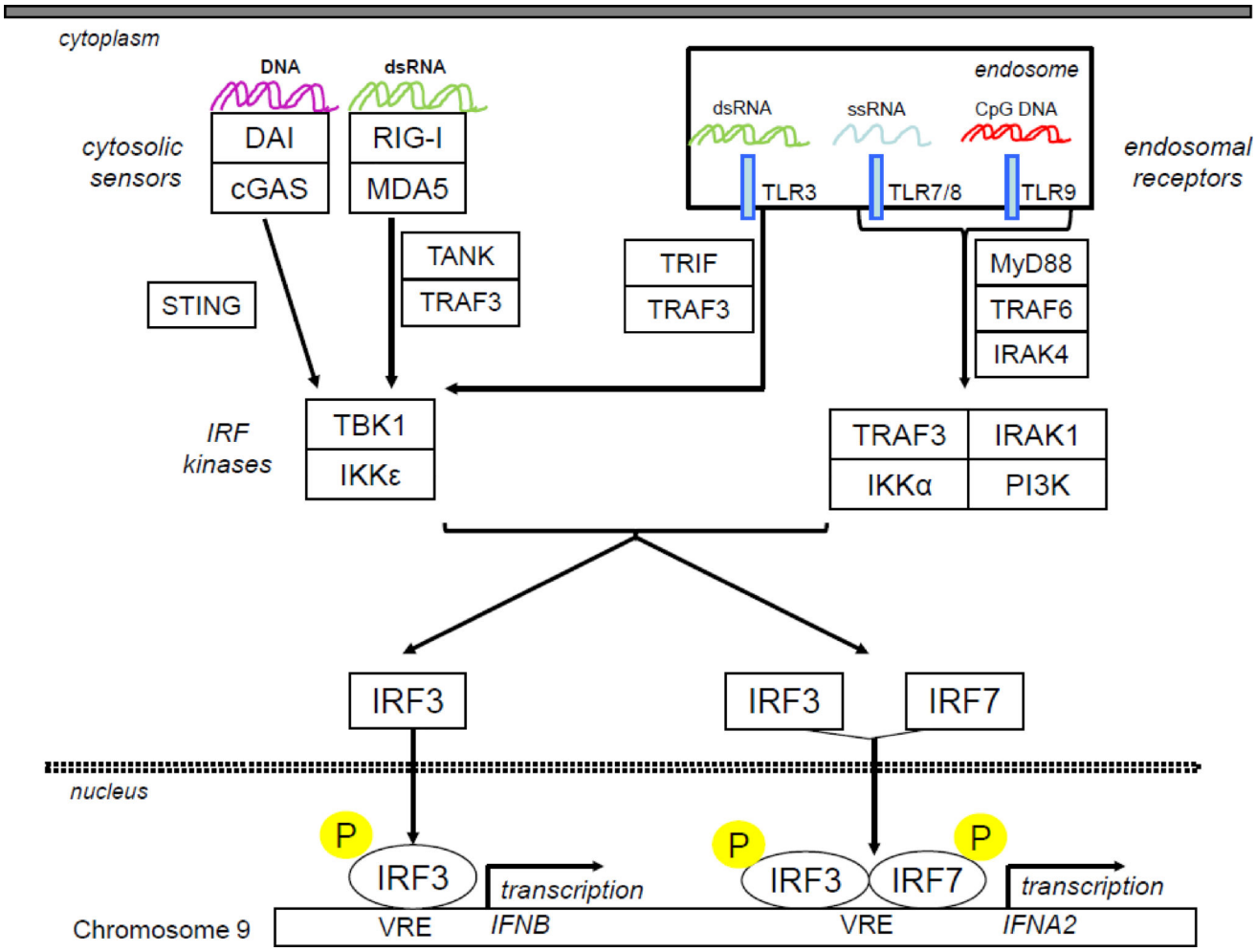

Figure 1. Induction of type I-IFNs

Damage and pathogen associated molecular patterns are recognized by cytosolic sensors and/or endosomal receptors which activate several pathways leading to the activation of IRF kinases and the subsequent phosporylation of IRF3 and IRF7.Once phosphorylated, these IRF translocate into the nucleus, bind to the promoter of type I IFN genes and activate their transcription. Unlike IFNB gene, IFNA2 gene requires both IRF3 and IRF7 activation to be induced. cGAS: Cyclic GMP-AMP synthase; CpG DNA: DNA with cytidine-phosphate-guanosine motifs; DAI: DNA-dependent activator of IFNregulatory factors; dsRNA: double strand RNA; IKK: inhibitor of kappaB kinase; IRAK: Interleukin-1 receptor-associated kinase; IRF: Interferon regulatory factors; MDA5: Melanoma Differentiation-Associated protein 5; MyD88: myeloid differentiation primary response protein 88; RIG-I: retinoic acid-inducible gene 1; STING: Stimulator of interferon genes; TBK: TANK-binding kinase; TLR: Toll-like receptor; TRIF: Toll/IL-1 receptor (TIR) domain-containing adaptor inducing *IFN*β, TRAF: TNF receptor associated factors; VRE: virus responsive element.

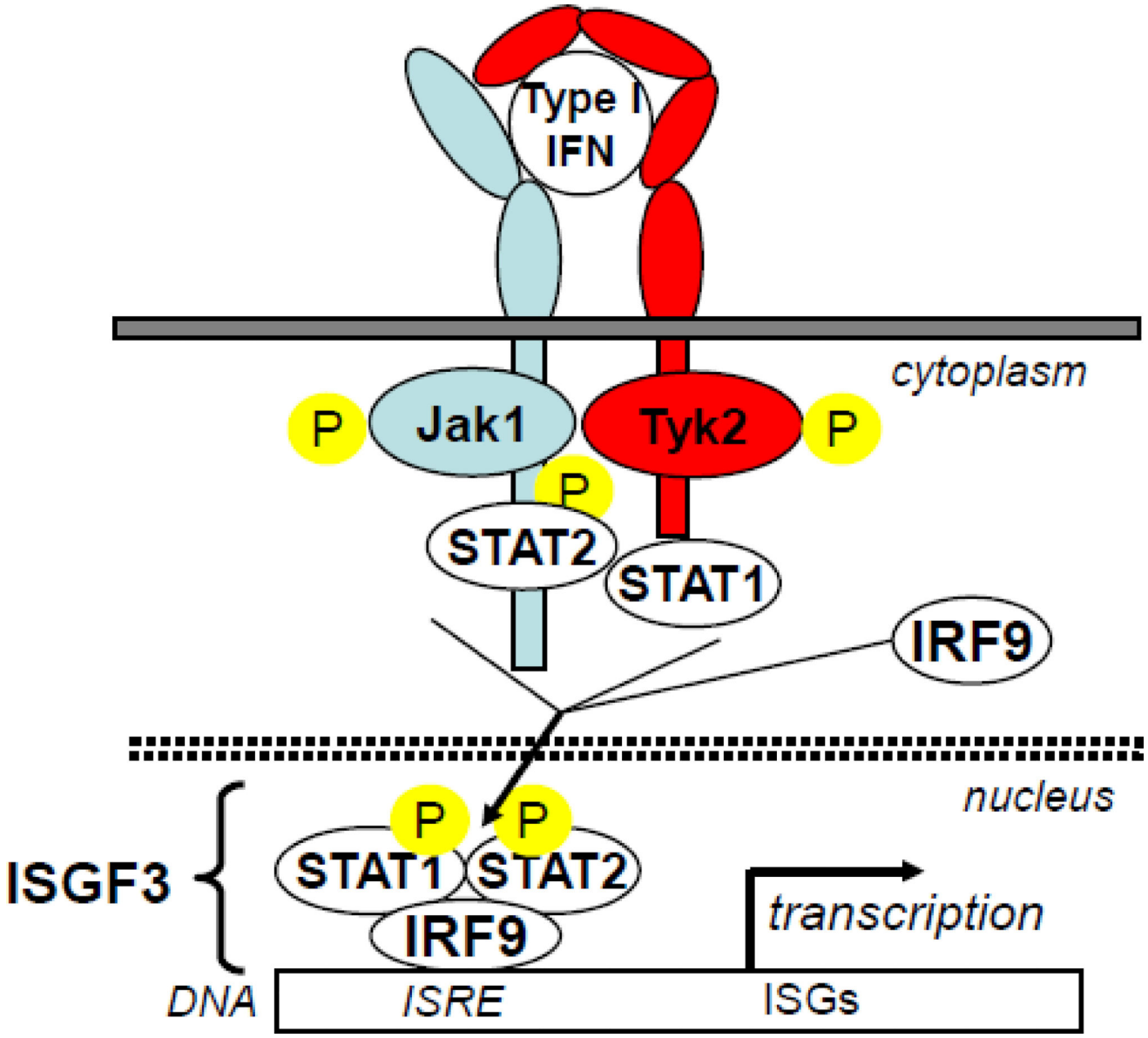

Figure 2. Canonical JAK/STAT signalling pathway of type I-IFNs

Type I-IFN/IFNAR2/IFNAR1 ternary complex induces transphosphorylation of receptor associated JAK kinases which in turn phosphorylate specific tyrosine residues on IFNAR1 and IFNAR2. These phosphorylated tyrosine residues are docking sites for signal transducers and activators of transcription (STAT) which are then recruited to the receptor complex and phosporylated (pSTAT) on specific tyrosine residue. pSTAT2 and pSTAT1 heterodimerize to form together with IRF9 the ISGF3 complex which translocates into the nucleus and binds to IFN stimulated responsive elements (ISRE) in the regulatory region of IFN-stimulated genes (ISG)