

TYPE I INTERFERON RECEPTORS –

BIOCHEMISTRY AND BIOLOGICAL FUNCTIONS

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OVERVIEW

The type I interferon (IFN) receptor (IFNAR) is comprised, as other cytokine receptors, of multiple components, in this case designated IFNAR1 and IFNAR2. However it is unique among cytokine receptors in the number of cognate ligands, including 13 IFN α subtypes, β , ω , ϵ , κ and others in some species. The type I IFN receptors are distinct from those required for the type II IFN γ (IFNGR1 and IFNGR2) and type III IFNs (IFNLR and IL10R β). Nevertheless, genes encoding a component of each type of IFN receptor, namely *IFNAR1*, *IFNAR2*, *IFNGR2* and *IL10R β* are located on human chromosome 21q22.1 in a cytokine receptor gene cluster, typical of functionally related genes.

Although IFNs were identified 50 years ago and the existence of IFN receptors 10 years later, it was 1990 when the first type I IFN receptor, now designated IFNAR1 was cloned. This was achieved utilizing human gene libraries expressed in murine cells and rescue of the definitive, species specific antiviral activity of human IFN α 8 (1). The *IFNAR2* cloning was achieved by firstly identifying a human IFN binding activity in urine, peptide sequencing, and then gene library

screening with derived oligonucleotides (2). It was subsequently discovered that the original cDNA encoded only one isoform of the *IFNAR2* gene, which also encoded a long transmembrane isoform that transduced a signal, a truncated transmembrane isoform and a soluble/secreted isoform (3) (Fig. 1A).

Subsequently, the functions of the type I IFN receptors have been elucidated with respect to ligand interaction, mechanisms of signal transduction and biological responses. The pioneering studies that discovered IFNARs and their mechanisms of actions *in vitro* have been largely validated *in vivo* using gene targeted mice. This body of work has highlighted the important roles of IFNARs in mediating type I IFN responses in haemopoiesis, innate and acquired immunity to infection and cancer. However, IFNs elicit many biological effects which can even be opposite in different cell types. For example, type I IFN inhibits proliferation and is pro-apoptotic for many cell types (4), yet prolongs the survival of memory T cells (5). Understanding the function of the IFNAR complex will elucidate how such a diversity of biological outcomes is generated.

GENES AND GENE EXPRESSION

The IFNAR genes encode multiple isoforms that contribute to the potential complexity of the functional receptor (2,3,6). Two splice variants of IFNAR1 were identified in cell lines (7,8). However, subsequent bioinformatic analyses of splice variants in Expressed Sequence Tag (EST) databases and Rapid Amplification of cDNA Ends (3' RACE) analyses from normal cells identified only one isoform, suggesting that the former are either artifacts or aberrant transcripts found only in particular tumor cell lines (Samarajiwa and Hertzog, unpublished data).

In contrast, four *IFNAR2* transcripts encoding three isoforms are generated from the same gene by exon skipping, alternative splicing and differential usage of polyadenylation sites (3) (Fig. 1A) These transcripts encode a long transmembrane IFNAR2c, a short transmembrane IFNAR2b chain and a soluble sIFNAR2a chain. Transfection of human IFNAR1 and IFNAR2c, but not IFNAR2b reconstituted the antiviral IFN response (9). This is consistent with data, at least in sarcomas, that IFNAR2b may act as a dominant negative regulator of IFN responses (10) (Fig. 1B).

The mouse has been the primary model for pathophysiological studies of IFNs due to the ability to generate knockout mice that can demonstrate cause-effect associations *in vivo*. The mouse has a comparable type I IFN system to human with multiple ligands (α 's, β , ϵ , etc.) and *Ifnar1* and *Ifnar2* genes (11,12). No IFNAR2b chain has been identified in mouse; but two transcripts capable of encoding soluble isoforms (*sIfnar2a* and *sIfnar2a'*) are generated by differential splicing. The more abundant 1.5kb *sIfnar2a* transcript encodes the complete IFNAR2 extracellular domain and reads-through the splice site on exon 7-7' boundary producing a transcript encoding 12 unique and mostly hydrophobic C-terminal residues (6). The *sIfnar2a'* minor transcript is generated from a transcript missing 128 nucleotides after codon 236 of the *Ifnar2* cDNA. This isoform originates by skipping the transmembrane-encoding exon 8, leading to a frame shift forming a stop codon that generates a transcript capable of producing a soluble receptor with 11 unique C-terminal amino acids (Fig. 1A).

Transcriptional Regulation

The transmembrane and soluble *Ifnar2* transcripts are differentially regulated based on northern blot analyses of expression in murine tissues. Ratios of *tmIfnar2c*: *sIfnar2a* range from >10:1 in some tissues to approximately 1:1 in haemopoietic tissues (11). Analysis of the 5' flanking region of *Ifnar2* using promoter reporter constructs identified three regulatory regions conferring basal expression, inducible expression by IFN α +IFN γ and a negative regulatory region (11-13).

STRUCTURE FUNCTION RELATIONSHIPS

Studies of type I IFN receptors prior to their cloning, indicated that most cell types bound IFNs, with large variation in the number of binding sites (200 - 10,000 per cell) and binding affinities. Scatchard analyses of binding usually identified two types of binding sites of low (μ M) and high affinity (nM-pM) (14). This pattern of binding is consistent with a multicomponent receptor containing a high affinity binding chain (often called the α or primary binding chain) and a β or signal transducing chain that had low intrinsic ligand binding affinity and converted the affinity of interaction of ligand with α chain from moderate (nM) to high affinity (pM) (15). Recently, elegant studies using recombinant IFNAR extracellular domains (ECDs) tethered to lipid membranes clearly demonstrated that various type I IFNs bind to IFNAR2 with K_d mostly in the nM range (from 0.1 to 1000nM) and bind to IFNAR1 with K_d mostly in the μ M range (from 0.05 to 10 μ M) (16).

IFNAR structures

IFNAR1 and IFNAR2 belong to the class II helical cytokine receptor (hCR) family, which includes the receptors for type II IFN, tissue factor (TF) and IL10R β (15). Members of the class II hCR family contain tandem \sim 100 amino acid (aa) domains with a predicted topology analogous to immunoglobulin (Ig) constant domain (15). The ECD of huIFNAR1, comprising 409 aa (403 in the murine form) contains four subdomains referred to as SD1 through SD4, each housing one Fibronectin (FBN-III-like) domain (Fig. 2). SD1 contains conserved residues implicated in binding

membrane glycosphingolipids (17). SD1-SD3 appears to house the ligand binding domain; SD4 is essential for ternary complex formation (18). The SD1-SD2 pair is structurally similar to the SD3-SD4 pair with characteristic disulfide bonding cysteine pairs (15) and 50% sequence homology (19).

NMR has been used to model huIFNAR2 structure and its interaction with huIFN α 2 (20) which has similarities and differences with the resolved crystal structures of two members of the class II hCR group, TFR (21) and IFNGR (22). As predicted for class II hCR receptors (15), all three proteins have two FBN-III domains with Ig-like folding topology and two conserved disulfide bonds (22-24). However, unlike TFR and IFNGR which show a conserved inter-domain angle of $\sim 120^\circ$ (21,22), huIFNAR2 has an interdomain angle approximating 90° (20) (Fig. 2B). Based on the prediction that type I IFNs have an interaction surfaces for each receptor on opposing sides of the ligand (23), and information regarding the locations of amino acids implicated in ligand binding on both IFNAR1 and IFNAR2, a three-dimensional model for the ligand-bound human type I IFN receptor complex has been proposed (24). This model predicts that upon ligand binding the N-terminal FBN-III domain of IFNAR1 forms a lid over the bound ligand (24). However, this prediction fails to consider the residues of IFNAR1 implicated in binding membrane glycosphingolipids (17) so the N-terminal of IFNAR1 may fold towards the membrane, stabilizing the molecule before or after ligand binding.

IFNAR Ligand Interaction

The binding site of IFN α 2 on IFNAR1 has been predicted from site-directed mutagenesis (24,25) and epitope mapping with an anti-IFNAR1 neutralizing antibody (26) (Fig. 2), albeit that the latter may be inaccurate due to steric hindrances. Residues involved in ligand binding are found on the three membrane-distal FBN-III SD domains. Residues ⁶⁹VY⁷⁰ have been identified as the key residues in IFNAR1 recognition by a neutralizing monoclonal antibody (26) and thus proposed to influence IFN α 2 binding (24). A number of other surface-exposed, aromatic residues within the

surrounding region have also been demonstrated to aid IFN α 2 binding (24) (Fig. 2).

Studies of our laboratory and others suggest that IFNAR1 is necessary for signaling and is possibly responsible for the differential recognition of the IFN ligands (16,18,27,28). Numerous studies have investigated residues of IFNAR2 involved in ligand interactions with IFN α 2 and IFN β (20,25,27,28) (Fig. 2). Notably, the ligand binding site of huIFNAR2 is composed largely of aliphatic hydrophobic amino acids (20-22) (Fig. 2). Forming the core of the IFNAR2 ligand binding domain are three highly conserved residues T⁴⁴, M⁴⁶ and K⁴⁸, while residues, H⁷⁶, E⁷⁷, Y⁸¹, W¹⁰⁰, I¹⁰³ and D¹⁰⁶ also facilitate IFN α 2 binding (27,28). These residues are predicted by NMR to form an extensive and largely aliphatic hydrophobic patch on the surface of IFNAR2 (Fig. 2) (20). The interaction of IFN β is predicted to be different to IFN α , involving IFNAR2 residues I⁴⁵ and W¹⁰⁰, with minor contributions from T⁴⁴, M⁴⁶, S⁴⁷ and I¹⁰³ (Fig. 2) (28). It appears that the sequence differences between type I IFNs result in different binding affinities with each IFNAR chain and consequent biological activities (16).

Soluble IFNAR

Soluble cytokine receptors are present in body fluids and modulate cytokine activity during homeostasis and disease (29). Soluble IFNAR2 receptors are present in serum, urine, saliva, peritoneal fluid of both humans and mice (11,30). *In vitro* studies demonstrate that a soluble IFNAR2 can also be generated by cleavage of transmembrane IFNAR2 by intra-membrane proteases in response to IFNs and other stimuli (31), but there is no definitive *in vivo* evidence for this. Nevertheless, there is a precedent in the IL6 receptor system where the soluble IL6R α is generated both by alternative splicing and by cleavage by ADAM 10 and 17 proteases (32).

While a definitive function for the soluble IFNAR2 isoform has not yet been resolved, *in vitro* experiments have demonstrated that sIFNAR2a can act either as an agonist or antagonist (11). Soluble IFNAR2a can inhibit IFN signaling in normal cells; while in primary

thymocytes from *Ifnar2^{-/-}* mice, sIFNAR2a can bind IFN α or β and generate an anti-proliferative signal (Fig. 1B). In another study, ovine soluble IFNAR2 was able to mediate antiviral activity *in vitro* (33). Transgenic mice over expressing the soluble receptor are more susceptible to LPS induced, IFN β mediated septic shock, suggesting that high levels of the sIFNAR2a receptor can potentiate IFN signaling *in vivo* (Samarajiwa et al., unpublished data). Potentiation or agonist actions of soluble IFNAR2a may be mediated by the process of trans-signaling (see below) which is the major method of signal transduction by sIL6R and sIL15R (32,34). Soluble IFNAR2a might also have a carrier function since IFN β bound sIFNAR2a increased the stability of IFN β and enhanced the anti tumor activity in a xenograft tumor model (35).

MECHANISMS OF SIGNALING

Signal transduction domains and pathways

The type I IFN receptor, typical of class II hCR, lack intrinsic kinase activity, and thus rely on associated Jak kinases to phosphorylate receptors and signal transducing molecules such as STAT proteins, after ligand-induced receptor clustering. IFNAR1 is pre-associated with Tyk2 (36) which also stabilizes IFNAR1 cell surface expression levels (37). The Tyk2 binding site on the huIFNAR1 cytoplasmic region has been localized to a region encompassing residues 479-511 (36). HuIFNAR1 also bound STAT1 and STAT2 via phospho-Y⁴⁶⁶ and phospho-Y⁴⁸¹ (38) when over expressed in heterologous cells (4). STAT3 reportedly undergoes phosphotyrosine-dependent interaction with IFNAR1 (39), consistent with STAT1 and STAT3 homo- and heterodimer formation after IFN α treatment (6).

Using truncation mutants of the intracellular domain of huIFNAR2, the site of Jak1 binding was identified to a 47 aa region (40). Jak1, STAT1, and STAT2 may also be pre-associated with IFNAR2 (41). This data suggests that the intracellular domains and signal transducing molecules such as STATs may form multimolecular signal transduction complexes in which each molecule has multiple interactions (42).

Further diversity of IFNAR signaling is achieved by the activation of other pathways including other STAT proteins and non-STAT proteins (4). These "alternative" signaling pathways include CrkL, Rap1, MAP kinases, Vav, RAC1, PI3kinase, IRS1 and 2, PMRT1 and Sin1 (4).

Negative regulation of signal transduction

The diversity of signals generated through IFNARs can protect the host against infection and cancer and mount controlled immune responses; whereas excessive or deregulated signaling can lead to toxicity, leucopenia, autoimmunity and even death. Thus, IFNARs also interacts with a number of negative regulatory molecules including Suppressor of Cytokine Signaling (SOCS-1), UBP43 and SHP (43-45) to limit the extent of signaling. The C-terminus of IFNAR1 contains a highly conserved region spanning 14 amino acids which mediates inhibition of type I IFN signaling (46). This may occur by binding a negative regulator such as SOCS1 to inhibit JAK/STAT signaling (45). Residues within this region are also essential for the recruitment of E3 ubiquitin ligases, and ubiquitination and degradation of the receptor (46). Recently, a type I IFN-inducible cysteine protease, UBP43, was shown to directly interact with IFNAR2 and block the interaction between Jak1 and the receptor (44).

Trans-signaling

Soluble cytokine receptors such as sIL6R, sCNTFR, sIL11R and sIL15R can mediate cytokine biological effects by a mechanism known as trans-signaling (Fig 1B). Trans-signaling occurs when the soluble receptor bound ligand interacts with a complementary transmembrane receptor chain of the receptor complex (32,34). In the case of IL6R α and the signaling gp130 chain, this is the major mechanism of IL6 signaling since gp130 is expressed on most cells, but transmembrane form of IL6R α shows restricted expression (47). We have demonstrated *in vitro* that sIFNAR2a can bind IFN α or β , and transduce a signal through IFNAR1 (11). Furthermore, our experiments using mice over-expressing sIFNAR2a suggests that high levels of sIFNAR2a may act as trans-signaling molecules *in vivo* (Samarajiwa et al., unpublished data). While more direct evidence of trans-signaling in the IFN system is required, it

provides a compelling mechanism for transducing alternative signals.

IN VIVO FUNCTION

Expression patterns

All tissues, organs and most cell lines express transcripts for IFNAR1 and both soluble and transmembrane isoforms of IFNAR2 (11-13) and thus bind and respond to IFNs. Microarray analysis of *Ifnar* expression in different tissues and cells indicate that there is some differential expression of IFNAR1 and IFNAR2. IFNAR1 being more widespread and IFNAR2 more restricted. These studies need to be validated by other techniques, with reagents that enable the measurement of IFNAR protein levels, particularly at the cell surface to enable a clearer picture of the composition of the IFNAR receptor on different cell types during homeostasis and disease.

Knockout studies

Mice with null mutations in *Ifnar1* demonstrated that this component was essential for responses to multiple IFN α as well as IFN β (48,49). Extensive use of these mice demonstrated *Ifnar1* necessity for survival against most viral infections, myelopoiesis and B and T cell mediated immune responses and was a potent proinflammatory cytokine.

We have also generated mice with a null mutation in the *Ifnar2* gene which had a similar phenotype to *Ifnar1* null mice, in their susceptibility to viral and bacterial infections, but distinct in their abnormal thymic T cell development (Hertzog et al., unpublished data). The subtle difference in phenotype were notable in their homeostatic role in haemopoiesis and in the mediation of proinflammatory signals (45). The mechanism and nature of these differential signals remain to be elucidated.

DISEASE ASSOCIATIONS

Polymorphisms in promoters and genes encoding type I IFN receptors have been implicated in a number of diseases. Protection or susceptibility against cerebral malaria (50), susceptibility to multiple sclerosis (51), Trypanosomiasis (52),

HIV (53), Hepatitis B and C virus (54,55) are influenced by IFNAR polymorphisms.

In Down syndrome when HSA 21, containing the IFNAR1 and IFNAR2 genes, is trisomic, cells are more sensitive to IFN α treatment and the aberrant immune response in this condition has been associated with aberrant IFN signaling (56). The levels of sIFNAR2a have not been assessed in this condition.

Increased levels of sIFNAR2a have been reported in many chronic viral infections, cancers and urological diseases (54,57,58). During chronic hepatitis C infection total *Ifnar2* transcript levels increased by over 10 fold as well as sIFNAR2a serum levels (58). The increased serum levels of sIFNAR2a correlated with increases in serum 2-5 OAS activity ($P < 0.001$) suggestive of potentiated IFN activity (58). Interestingly other studies did not find a correlation between increased sIFNAR2a serum levels and lack of response to IFN therapy. While the importance of soluble IFNAR2 in human disease will be clearer once it is ascertained whether it mostly participates in agonist or antagonist activities, the above studies support the former.

Not only do host cells produce IFNAR proteins during viral infections, but certain viruses have evolved a form of soluble type I IFN receptor as a means of evading the immune response. Poxvirus encode a soluble IFN receptor homologue that neutralizes all type I IFNs tested and this is essential for virulence (59). This is an unusual protein that has low amino acid homology to IFNAR1 or IFNAR2, but does have tertiary structural similarity based on modeling studies, and is a potent inhibitor of the antiviral activity of a broad range of type I IFNs without the species specificity of mammalian IFN-IFNAR interactions.

SUMMARY AND PERSPECTIVE

The IFNAR complex is novel among cytokine receptors in mediating signaling by more than 15 different, but related type I IFN ligands. This system has been instrumental in the discovery of the JAK-STAT signaling pathway which is necessary for regulating genes involved in the

characteristic antiviral response. However emerging data indicates that many more so-called “alternative” pathways are activated by IFNAR activation. This diversity of signals may explain how IFNs generate complex biological responses. There has been considerable advance in understanding the structure of the receptor signaling complex. However, more detailed structural studies and confirmation of the potential different receptor configurations like that which would elicit trans-signaling, are necessary to

elucidate how different biological activities of type I IFNs can be regulated by the receptor.

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FIGURE LEGENDS

Figure 1

A. Differential splicing of human and murine *Ifnar2*

(i) Murine *Ifnar2* generates one transcript encoding a long *Ifnar2c*, while two soluble specific transcripts are generated by either exon skipping (*sIfnar2a'*) or read through into intron 8 (exon 7') (*sIfnar2a*).

(ii) Human IFNAR2 is alternatively spliced to generate transcripts encoding a long isoform (*IFNAR2c*) similar to the mouse, a short isoform (*IFNAR2b*) and soluble (*sIFNAR2a*) isoforms by exon skipping. Soluble *IFNAR2a* is generated by splicing at exon 7 into splice acceptor site (sa1) within exon 9 and uses poly A site 1 (*1). The long *IFNAR2c* uses exon 7, 8 and sa2 (*2). Short *IFNAR2b* uses exon 7, 8 and either poly A sites *1 or *2.

B. Receptor complexes and IFN signaling

1. Conventional signaling occurs when IFN binds to IFNAR1 and tmIFNAR2c resulting in cross-phosphorylation of receptors and associated janus kinases (Tyk2 and Jak1). This provides docking sites on the receptor complex for STAT proteins. STAT proteins are in turn phosphorylated and form homo and heterodimeric complexes which dissociate from the receptor, then translocate to the nucleus and bind to ISRE or GAS element within the promoters of interferon regulated genes, leading to their transcription.

2. IFNAR2b acts as a dominant negative modulator of IFN signaling by binding ligand but not transducing antiviral signals.

3. Trans-signaling by ligand bound soluble IFNAR2a interacting with IFNAR1 can generate a biological response.

Figure 2. The type I IFN receptor signaling complex.

The three components, huIFNAR1ECD (left), huIFN α 2 (middle) and huIFNAR2ECD (right) were modeled using Rasmol and are given at approximate relative size. The amino (N) and carboxyl (C) terminals of each component and the four subdomains (SD) of IFNAR1ECD are indicated. The huIFNAR1ECD model (left, Spacefill format) was predicted from the crystal structure of human fibronectin (PDB reference 1FNF). Amino acid residues of IFNAR1ECD that reportedly interact with membrane-bound glycosphingolipids are shown in yellow, and those that are important for huIFN α 2 binding in violet. The huIFN α 2 structure (middle, Ribbon format) was determined by NMR (PDB reference 1ITF). Amino acid residues involved in IFNAR1 interactions are shown in green (Stick format) and those involved in IFNAR2 interactions shown in violet (Stick format). The huIFNAR2ECD structure (right, spacefill format) was determined by NMR (PDB reference 1N6U) and shows amino acid residues that are important for huIFN α 2 binding (red), huIFN β (green) and those residues that are important for binding of both ligands (orange).

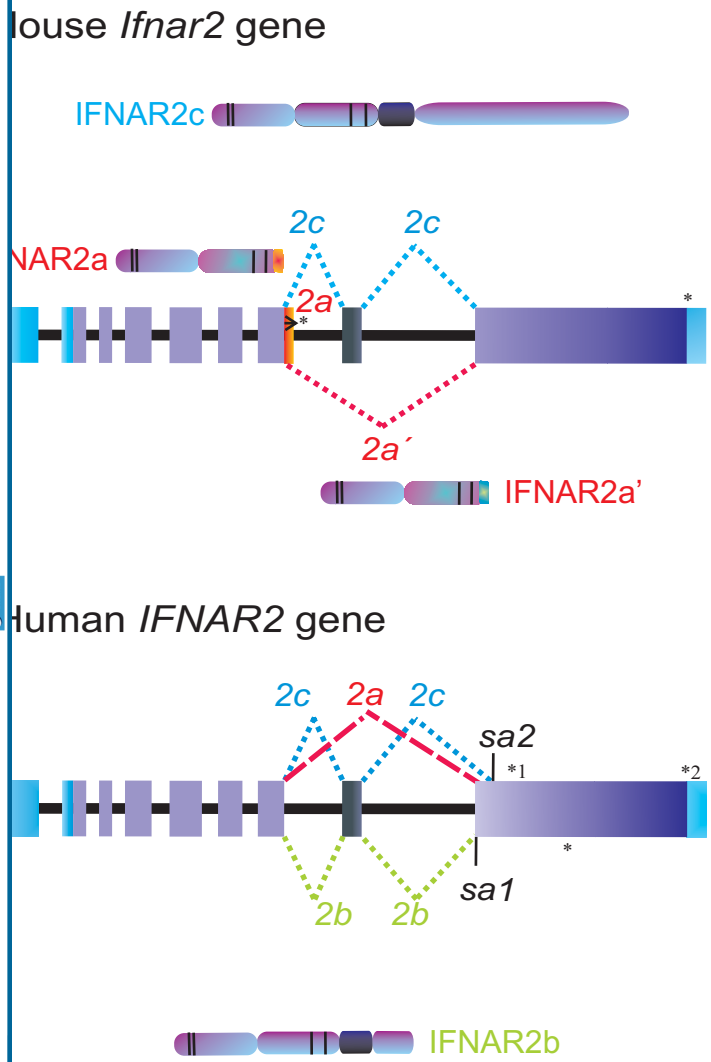
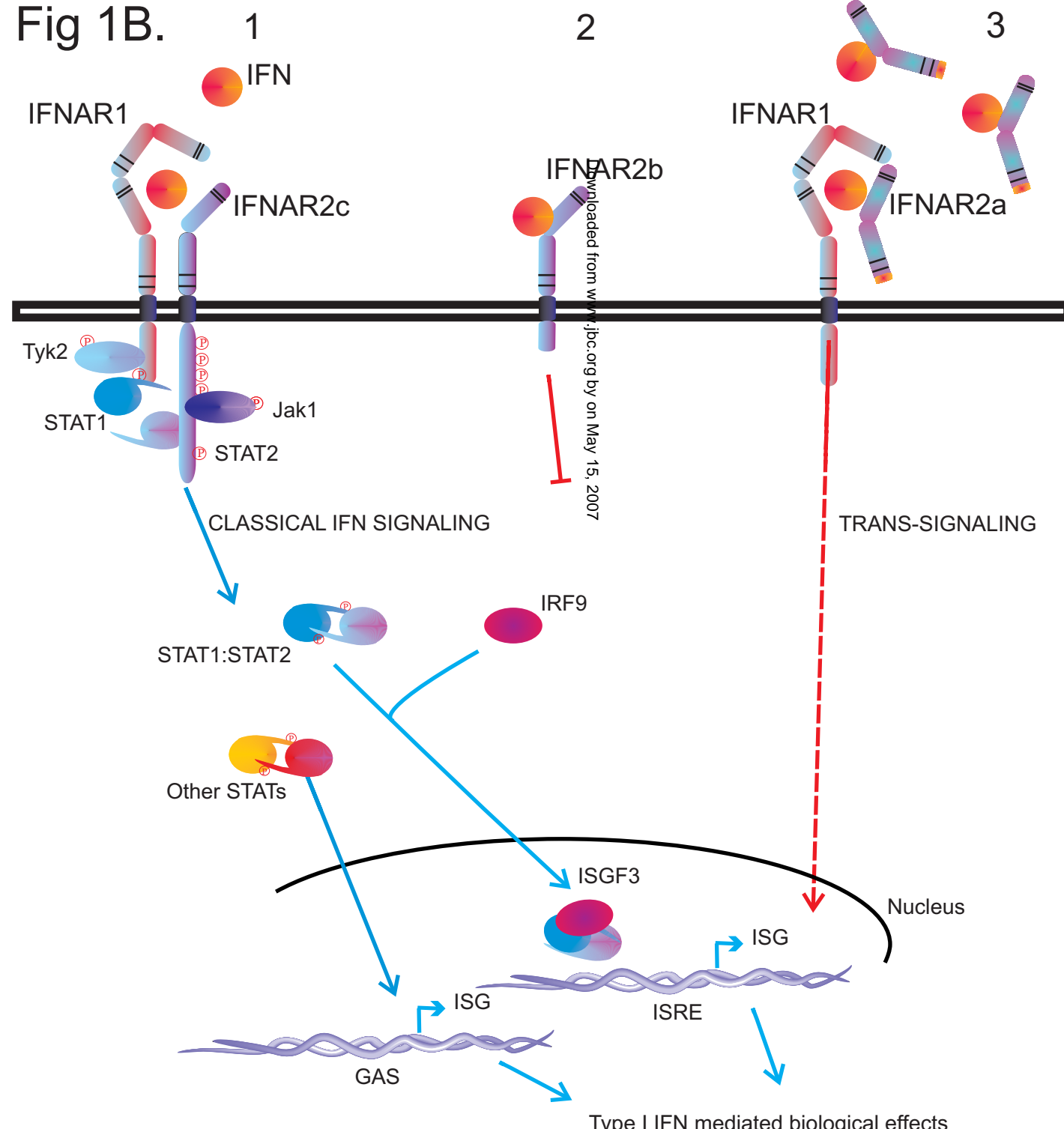


Fig 1B.



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