# 1. Introduction

## 1.1 Cytokines and interferons

Cytokines play an important role in the communication between cells of multicellular organisms. The typical cytokine is a small glycosylated monomeric peptide of about 150 amino acids (Callard and Gearing, 1994). Most cytokines are secreted from the cells, forming soluble messenger proteins. They act by binding to specific membrane receptors, which then transmit the signal into the cell, ultimately leading to changes in gene expression. Cytokines generally act over short distances, being autocrine (acting on the cell that produced them), or paracrine (acting on cells close by) rather than endocrine (acting on cells at a distance). Cytokines have been classified according to their biological response into pro- and anti-inflammatory cytokines, according to the receptors used or according to their three-dimensional structures (Nicola, 1994). Most prominently, cytokines are involved in signalling between cells during the immune response. They are extremely potent, acting at picomolar and sometimes even femtomolar concentrations. Cytokines are part of an extracellular signalling network that controls every function of the immune system: inflammation, defence against virus infection, as well as proliferation and differentiation of lymphocytes.

An important class of cytokines are the interferons. They have been discovered due to their antiviral activities and protect uninfected cells from viral infection (Isaacs and Lindenmann, 1957). There are two types of IFNs, termed type I and type II interferons, and interferon-like cytokines. Type I interferons (IFNα, -β, -δ, -ε, -κ, -τ and -ω) are produced by virtually any virus-infected cell, and provide an early innate immune response against viruses. They induce uninfected cells to produce enzymes capable of degrading mRNA. Type I interferons confer resistance against many different viruses, inhibit proliferation of normal and malignant cells, impede multiplication of intracellular parasites, enhance macrophage and granulocyte phagocytosis, augment natural killer (NK) cell activity, and show several other immunomodulatory functions (Stark et al., 1998; Pestka et al., 2004). The type II interferon (IFNγ) is structurally unrelated to type I interferons. It is mainly produced by activated T cells and NK cells. It conveys protection against viral infection in a later antigen-specific phase phase of the immune response (adaptive immune response). Apart from their antiviral activities interferons also possess antiproliferative and immunomodulatory activities and influence the metabolism, growth and differentiation of many different cells.

## 1.2 The JAK-STAT pathway

The central mediators in the response of cells to interferons are the signal transducer and activator of transcription (STAT) proteins. They were named STATs to denote their ability to serve as both cytosolic transducers of extracellular signals and as nuclear transcription factors (Darnell et al., 1994). In unstimulated cells STATs predominantly reside in the cytoplasm. Binding of cytokines or growth factors to their cognate receptors initiates a series of phosphorylation events carried out by members of the Janus family of kinases (JAKs), which leads to the phosphorylation of STATs on a single tyrosine residue (Fig. 1.1). This process, commonly termed STAT 'activation', triggers the dimerisation and nuclear translocation of the STAT proteins. In the nucleus STATs bind to specific recognition sequences in the promoters of their target genes and activate transcription, thereby directly converting a stimulus at the cell surface to an alteration of the genetic program (Darnell, 1997; Stark et al., 1998; Levy and Darnell, 2002). The mechanism how STATs gain access to the nucleus and how this nucleocytoplasmic transport is regulated has become an area of intensive research (McBride and Reich, 2003; Vinkemeier 2004).

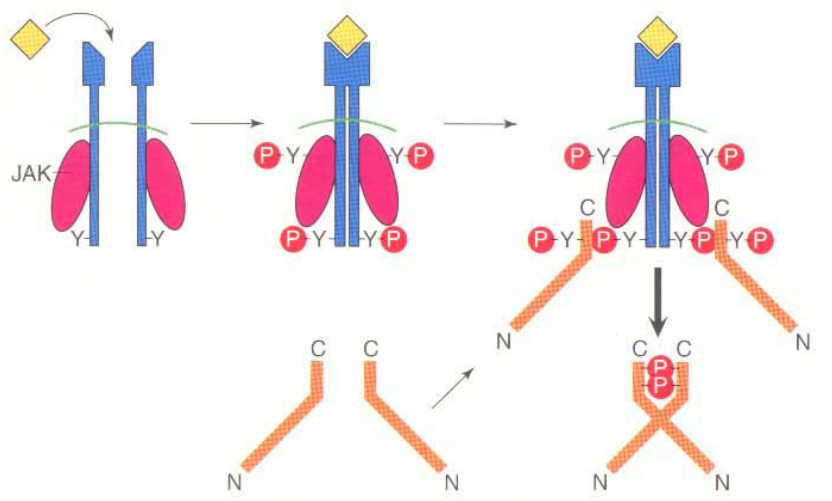


**Fig. 1.1** General model of STAT activation. Ligand (yellow diamond) binding induces receptor (blue) oligomerisation and activation of JAK kinases (red) that phosphorylate (P) each other and the receptor (blue) cytoplasmic domain on tyrosine residues (Y). Cytoplasmic STAT protein (orange) SH2 domains recognise specific receptor phosphotyrosine residues, and the receptor-bound STATs become phosphorylated, dimerise, and translocate to the nucleus where they bind to promoter elements (adopted from Horvath, 2000).

The best understood pathway is the activation of STAT1 in response to IFNγ. This type II interferon is the principal cytokine for activating macrophages. It also induces the

production of major histocompatibility complex (MHC)-I molecules, MHC-II molecules, and co-stimulatory proteins by antigen presenting cells (APCs). Furthermore, IFNγ promotes the cell-mediated immunity and increases the antimicrobial and tumoricidal activity of monocytes, macrophages, neutrophils, and NK cells. Genetic and biochemical analyses have revealed the importance of the protein tyrosine kinases JAK1 and JAK2 and of the transcription factor STAT1 in IFNγ-dependent signalling (Darnell et al., 1994; Darnell, 1997). Upon ligand binding, the IFNγ receptor subunit 1 (IFNγR1) and subunit 2 (IFNγR2) oligomerise and JAK1 and JAK2 are activated, leading to the phosphorylation of tyrosine 440 of the IFNγ receptor subunit 1. Phosphorylated $Tyr^{440}$ provides a docking site for STAT1 (Greenlund et al., 1995), which is in turn phosphorylated on tyrosine 701 (Schindler et al., 1992; Shuai et al., 1993), leading to its homodimerisation and accumulation in the nucleus. Here STAT1 dimers bind to palindromic DNA sequences with the general consensus $TTCN_{2-4}GAA$ (where N can be any nucleotide), termed gamma-activated sequences (GAS, Decker et al., 1997), within the promoters of target genes thereby regulating their expression (Shuai et al., 1992 and 1994; Darnell, 1997).

In response to type I interferons (IFNα, -β, and -ω) STAT1 primarily forms the complex transcription factor ISGF3 (interferon-stimulated gene factor 3), which additionally includes STAT2 and IRF9/p48 and binds to interferon-stimulated response elements (ISREs, Levy et al., 1988) (Ihle, 2001). In addition, STAT1 can also be activated by other cytokines and growth factors such as epidermal growth factor (EGF) or platelet-derived growth factor (PDGF) (Fu and Zhang, 1993; Wen et al., 1995a; Horvath, 2000). STAT1 knock-out mice are viable and display normal development. They specifically lack responsiveness to IFNα or IFNγ and are highly sensitive to viral infection (Meraz et al., 1996; Durbin et al., 1996). Similarly, germline mutations in human STAT1 have been reported to result in enhanced susceptibility to mycobacterial infection and lethal viral disease (Dupuis et al., 2001, and 2003). Furthermore, STAT1-deficient mice develop tumors when challenged with mutagens much more rapidly than wild type mice, which has implicated STAT1 as a tumor suppressor in an IFNγ-mediated tumor surveillance system (Kaplan et al., 1998). In addition, work carried out in tissue culture systems on STAT1-deficient cells demonstrated an important role for STAT1 in ligand-mediated growth arrest and apoptosis (Chin et al., 1997; Kumar et al., 1997; Su et al., 1997).

## 1.3 STAT proteins

STATs are conserved during evolution and have also been discovered in lower eukaryotes such as *Dictyostelium* and *Drosophila*, demonstrating the importance of STAT proteins in gene regulation (Darnell, 1997; Hou et al., 2002). Seven mammalian STAT genes have been identified: Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b and Stat6. The analysis of knock-out animals has established their functions in response to extracellular cytokine and growth factor signals in a wide range of cell types and tissues (Darnell, 1997; Akira, 1999; Ihle, 2001). STATs share a common domain structure (Fig. 1.2). They contain a coiled-coil domain, which is involved in protein protein interactions, a central DNA binding domain, a src-homology 2 (SH2) domain, required for dimerisation, and a conserved tyrosine phosphorylation site ($Tyr^{701}$ in STAT1).

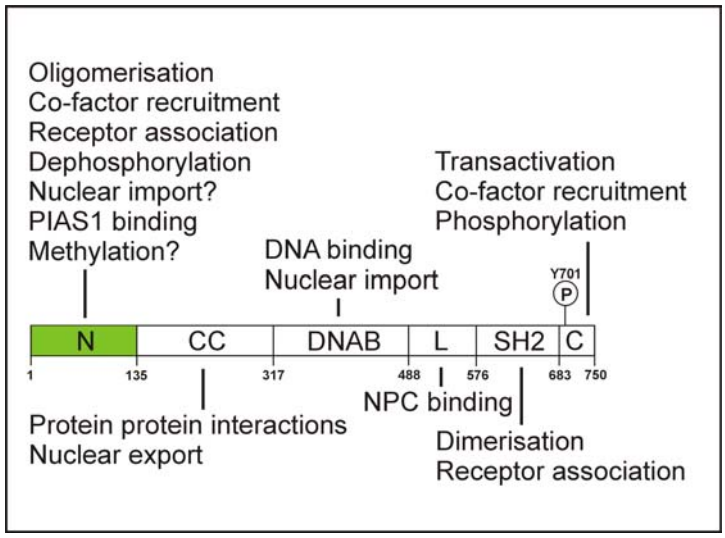


**Fig. 1.2** Functions attributed to the different domains of human STAT1. The domain structure of human STAT1 is depicted schematically. Numbers are the corresponding amino acid residues. N (N-domain); CC (coiled-coil domain); DNAB (DNA binding domain); L (linker domain); SH2 (src-homology 2 domain); C (C-terminal transactivation domain).

The very N- and C-terminal domains can be referred to as the ‘business ends‘ of the protein. The highly conserved N-terminal domain has been implicated in such diverse functions as oligomerisation, dephosphorylation and co-factor recruitment (see 1.4.2). The C terminus represents a transcriptional activation domain and has been shown to be involved in co-factor recruitment. Additionally, the C terminus is the site of an another post-translational modification: serine phosphorylation is required for maximal transcriptional activation (Decker and Kovarik, 2000; Varinou et al., 2003). Only recently, a function has been

attributed to the linker domain, which seems to be required for contacting the nuclear pore complex (NPC) during nuclear transport (Marg et al., 2004).

## 1.4 The STAT N-domain

*1.4.1 Structure of the STAT N-domain*

The $NH_2$-terminal domain of the mammalian STAT proteins (N-domain) comprises the first ~135 amino acids, and is highly conserved between the different STATs (Darnell, 1997). The N-domain represents an independently folding protein domain and is linked by a proteolytically cleaveable peptide to the large C-terminal STAT core domain, which contains the DNA binding and dimerisation (SH2-) domains (Vinkemeier et al., 1996; Chen et al., 1998). The structure of the N-domain of murine STAT4 has been resolved by crystallisation at a resolution of 1.45 Å (Vinkemeier et al., 1998; PDB ID 1BGF). It reveals a unique, overall α-helical fold with a hook-like appearance. Three structural elements can be distinguished: The $NH_2$-terminal 40 residues encompass four helices and form a ring-shaped element. An anti-parallel coiled-coil of helices α6 and α7 (residues 50-95) follows, and the last helix α8 docks to the distal surface of the ring-shaped element (Fig. 1.3).

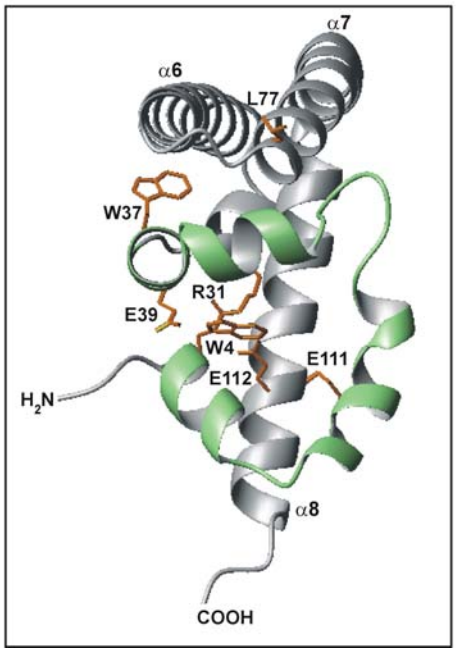


**Fig. 1.3** Structure of the N-domain of STAT4 (adopted from Vinkemeier et al., 1998). Side chains of residues mentioned in the text are depicted in orange. Residue Phe[77] of STAT1 is a leucine in STAT4. The helices 1-4 of the ring-shaped element are highlighted in green. Helices α6, α7, and α8 are labelled. The figure was prepared with the program MolMol using the PDB coordinates of the STAT4 crystal structure (1BGF).

The N-domain has a well-defined hydrophobic core which is conserved between the different STATs, consistent with a stable and defined fold. However, stabilisation of the most $NH_2$-terminal ring-shaped element involves unusual polar interactions including buried, solvent-inaccessible charges (calculated using a 1.4 Å probe radius). The ring is closed off by an α-helix-dipole interaction between the carboxylate group of $Glu^{39}$ and the $NH_2$-terminal part of helix α1 (see Fig. 1.3). $Glu^{39}$ is positioned correctly for this charge dipole interaction by the side chain of $Arg^{31}$ which forms an ion pair with $Glu^{112}$, which is in turn oriented by interactions with $Tyr^{22}$ and a buried water molecule. Interestingly, each of the side chains involved in stabilising the ring-shaped element is invariant in all STATs (see Fig. 1.4), suggesting that its architecture is conserved and may also reflect functional similarities (Vinkemeier et al., 1998).

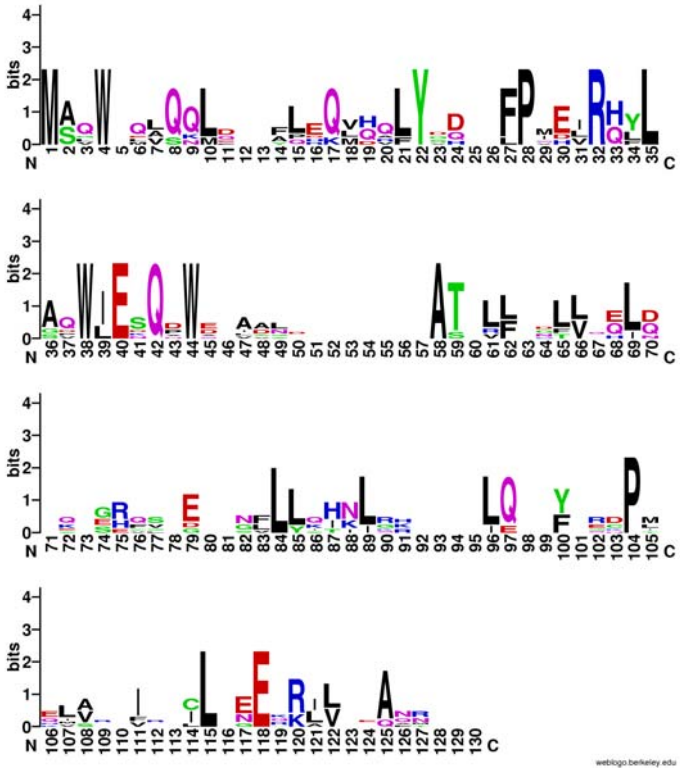


**Fig. 1.4** Sequence alignment showing the conservation of single amino acid residues between the $NH_2$-terminal domains of the 7 human STAT family members. The sequence alignment was prepared with ClustalW 1.8 and depicted using Weblogo (Crooks et al., 2004). The overall height of each stack of letters indicates the sequence conservation at that position (measured in bits), whereas the height of symbols within the stack reflects the relative frequency of the corresponding amino acid at that position. The numbering of the amino acids is according to that of the consensus sequence, the conserved $Arg^{31}$ of STAT1, thus corresponding to $Arg^{32}$; the STAT1 residues $Trp^{37}$, $Glu^{39}$, and $Glu^{112}$ can be found at the positions 38, 40, and 118 of the consensus sequence, respectively. Positively charged residues are given in blue, negatively charged residues in red, polar side chains in green and aliphatic residues in black.

*1.4.2 Functions of the STAT N-domain*

The STAT N-domain is a multi-functional protein domain that has been implicated in numerous protein-protein interactions (Shuai, 2000). One characteristic property is the ability of N-domains to form homotypic interactions that stabilise the formation of higher order STAT complexes. The N-domain of STAT4 forms homotypic dimers in crystals as well as in solution (Vinkemeier et al., 1998; Baden et al., 1998), and tetramerisation of two activated STAT dimers via N:N-domain interactions has been shown to be required for the cooperative binding of STAT dimers to tandem sites on DNA (Vinkemeier et al., 1996; Xu et al., 1996). Such oligomerisation via N:N-domain interactions leads to a prolonged half-life of the protein-DNA complex, and also allows binding to neighbouring GAS-like promoter elements, thereby extending the repertoire of potential STAT binding sites (John et al., 1999; Meyer et al., 2004). The crystal structure of the STAT4 N-domain revealed two alternative packing orientations of the N-domain dimers. The functionally relevant packing interface was mapped by mutational analysis and the conserved $Trp^{37}$ was identified as crucial residue in stabilising the interaction between two N-domains (Vinkemeier et al., 1998; John et al., 1999). N-domain-mediated cooperative DNA binding has in the meantime been confirmed for STAT1, STAT4, STAT5, and STAT3 (Vinkemeier et al., 1996; Xu et al., 1996; John et al., 1999, Zhang and Darnell, 2001).

Most prominently, the N-domain was attributed a role in the inactivation of STATs (Shuai et al., 1996). It has previously been observed that N-terminally-mutated forms of STAT1 are constitutively tyrosine phosphorylated following cytokine stimulation of cells (Shuai et al., 1996; Haspel and Darnell, 1999). Since analysis of the cytoplasmic to nuclear flow of activated STAT1 indicated that STAT1 is inactivated in the nucleus (Haspel et al., 1996; Haspel and Darnell, 1999), it was postulated that the N-domain is required for the recruitment of a nuclear phosphatase (Shuai et al., 1996; Haspel and Darnell, 1999). The nuclear phosphatase has recently been identified as the nuclear isoform TC45 of the T-cell protein tyrosine phosphatase (TC-PTP). TC45 can dephosphorylate STAT1 *in vitro* and *in vivo* and TC-PTP null mouse embryonic fibroblasts showed a prolonged STAT1 phosphorylation (ten Hoeve et al., 2002). Arginine 31 of the N-domain was suggested to directly contact the nuclear phosphatase, since mutation of this residue resulted in a similar hyperphosphorylation phenotype as observed upon deletion of the N-domain (Shuai et al., 1996). However, the crystal structure of the STAT4 N-domain revealed that $Arg^{31}$ is not surface-exposed. Rather it is located at the centre of the unusual salt-bridge arrangement that presumably stabilises the ring-shaped element (Vinkemeier et al., 1998 and Fig 1.3), thus

making it unlikely that $Arg^{31}$ directly participates in phosphatase recruitment without major structural rearrangements.

In addition, the STAT1 N-domain has been implicated in nuclear import of STAT1 (Strehlow and Schindler, 1998). Transport across the nuclear membrane can only occur via the nuclear pore complex (NPC, Ryan and Wente, 2000). The NPC forms an aqueous channel across the nuclear double membrane. The diameter of the channel is approximately 45 nm, the NPC thus represents a size selective filter (Görlich and Kutay, 1999; Macara, 2001). Only small molecules can enter or exit the nucleus by passive diffusion. The vast majority of macromolecules larger than ~40-60 kDa have to be actively transported in a carrier-assisted way (Görlich and Mattaj, 1996; Komeili and O'Shea, 2001). Depending on the directionality of the transport, the carriers are referred to as importins or exportins (Wozniak et al., 1998; Weis, 2003). Proteins to be imported into the nucleus are characterised by the presence of nuclear localisation signals (NLSs) which are recognised by members of the importin-α family of transport receptors (Dingwall and Laskey, 1991; Conti et al., 1998; Fontes et al., 2000). The best characterised "classical" NLSs are rich in the basic amino acids lysine and arginine and can exist either as monopartite or as bipartite clusters spaced by ~10 non-conserved amino acids (Dingwall et al., 1982; Kalderon et al., 1984; Lanford and Butel, 1984). Strehlow and Schindler (1998) showed that N-terminal mutants of STAT1 were unable to accumulate in the nucleus upon cytokine treatment. Nuclear accumulation of STATs, however, has been recognised as a process made up of two components: nuclear import, mediated by the Ran/importin-α system (Sekimoto et al., 1996 and 1997) and nuclear retention due to DNA binding (Meyer et al., 2003). The mechanism how the N-domain contributes to STAT nuclear accumulation has remained elusive. No classical nuclear localisation signal (NLS) could be identified in the N-domain so far (Sekimoto et al., 1997; Strehlow and Schindler, 1998), and N-terminal deletion mutants showed no other abnormalities, as they are activated normally, dimerise and are able to bind DNA *in vitro* (Strehlow and Schindler, 1998). During the course of this work, a dimer-specific NLS (dsNLS) has been identified in the DNA binding domain of STAT1 (Melen et al., 2001; Meyer et al., 2002a) and nuclear import of the activated STAT1 dimer was shown to be mediated by the import receptor importin-α5 (Fagerlund et al., 2002; McBride et al., 2002; Melen et al., 2003). However, the contribution of the STAT1 N-domain to importin-α5 binding has not been analysed.

Other functions of the N-domain have also been suggested, including receptor association (Li et al., 1997; Murphy et al., 2000), co-factor recruitment such as the CREB-

binding protein (CBP/p300, Zhang et al., 1996), and binding to the protein inhibitor of activated STAT1 (PIAS1), which blocks the DNA binding capacity of tyrosine phosphorylated STAT1 in an unknown way (Liu et al., 1998; Liao et al., 2000). Recently, it was proposed that the interaction of STAT1 and PIAS1 is regulated by arginine methylation of the STAT1 N-domain (Mowen et al., 2001).

*1.4.3 Arginine methylation of STAT1*

Protein arginine methylation is a post-translational modification whereby methyl groups are added to the guanidino group of arginine residues (McBride and Silver, 2001). It is catalised by protein arginine methyltransferases (PRMTs) that fall into two classes based on the symmetry of their reaction products (Gary and Clark, 1998). Both type I and type II enzymes generate $N^G$-monomethyl-arginine (MMA). However, whereas type I protein arginine methyltransferases (PRMT1, PRMT2, PRMT3, CARM1) account for the formation of assymmetric $N^G$,$N^G$-dimethyl-arginine (aDMA), type II enzymes (PRMT5) catalyse the formation of symmetric $N^G$,$N^G$-dimethyl-arginine (sDMA). Although the modification is well known, the biological role of arginine methylation as well as its regulation remain largely unknown. In the past, arginine methylation was mainly observed on abundant proteins such as histones and RNA binding proteins (McBride and Silver, 2001). A recent proteomic analysis has extended the repertoire of proteins that are modified by arginine methyltransferases, indicating its participation in many cellular processes (Boisvert et al., 2003). Evidence for a role of arginine methylation in cytokine signalling comes from two reports that described the physical interaction of PRMTs with components of the JAK-STAT pathway. PRMT1 has been shown to bind to the intracellular domain of the IFNαR1 and antisense abolition of PRMT1 resulted in alterations of the antiproliferative effects of IFNβ (Abramovich et al., 1997). Moreover, the most divergent protein arginine methyltransferase PRMT5 (also called Janus kinase binding protein 1, JBP1) was identified in a yeast two-hybrid screen as binding partner for JAK2 (Pollack et al., 1999). However, the targets of the associated arginine methyltransferases remained elusive.

In the year 2001, it was reported that the N-domain of STAT1 is targeted by the protein arginine methyltransferase PRMT1. David and co-workers presented evidence that arginine methylation of STAT1 is crucial for interferon (IFN)-induced transcription (Mowen et al., 2001). Strikingly, for efficient induction of IFN-inducible target genes, mono- and dimethylation of STAT1 was required, since blocking of STAT1 transmethylation prevented the induction of STAT1-dependent target genes (Mowen et al., 2001). The authors raised the

intriguing hypothesis that the IFN-unresponsiveness of multiple tumor cells, which are frequently found to accumulate endogenous methyltransferase inhibitors, might result from a concomitant block of STAT1 methylation. These ramifications are potentially of great clinical significance, because the growth retarding effects of IFNs are already exploited for cancer therapies and are an area of intense investigation (Lens et al., 2002; Chada et al., 2003). The authors identified the invariant arginine in position 31 of the conserved N-terminal domain as the methyl-acceptor (Mowen et al., 2001). Mono- and dimethylation of this residue or its mutation to alanine were both reported to boost growth arrest and transcriptional activity in response to IFN treatment of cells. On the molecular level, reduced binding of STAT1 to the inhibitory PIAS1 protein (Liu et al., 1998) under condition of arginine methylation was shown to improve DNA binding and thus transcription (Mowen et al., 2001; Fig 1.5).

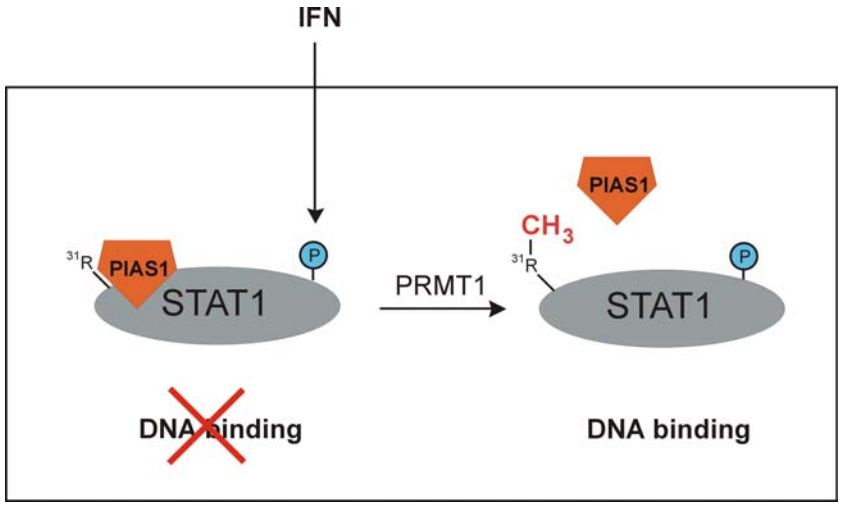


**Fig. 1.5** Model of STAT1 arginine methylation (deduced from the data of Mowen et al., 2001). In the absence of arginine methylation, the protein inhibitor of activated STAT1 (PIAS1) binds to phosphorylated STAT1 (P) and suppresses DNA binding of the transcription factor. The protein arginine methyltransferase 1 (PRMT1) was suggested to methylate ($CH_3$) the conserved arginine residue 31 ($R^{31}$) of the STAT1 N-domain leading to the dissociation of PIAS1 and STAT1. Only methylated STAT1 ($CH_3$) is able to bind to DNA and to act as a transcriptional activator.

The report of Mowen et al. (2001) has gained significant attention, since STAT1 was the first example of a mammalian signalling protein whose activity appeared to be regulated by arginine methylation and also because of the direct clinical relevance of their findings. However, our inital analysis of the STAT1 N-domain and its role in nuclear import of the transcription factor casted doubts on the above model (Fig 1.5). Therefore, a re-investigation of STAT1 arginine methylation and its impact on cytokine-induced gene expression was included in this work.

## 1.5 Ratjadone A – a putative nuclear export inhibitor

During the analysis of nucleocytoplasmic transport of STAT1 performed in this work, the cytotoxin ratjadone A was used to inhibit nuclear protein export. Ratjadone A was originally isolated as a secondary metabolite from the myxobacterium *Sorangium cellulosum* (Gerth et al., 1995; Fig. 1.6A). It displayed antifungal activity and was also shown to inhibit the growth of mammalian cell lines (Gerth et al., 1995; Burzlaff et al., 2003; for a review on ratjadones see Kalesse et al., 2001). Its mode of action and the cellular targets of ratjadone A, however, were unknown. Yet, its similar structure to the well characterised nuclear export inhibitor leptomycin B (LMB, see Fig 1.6B) suggested that both drugs may act through a common reaction mechanism.

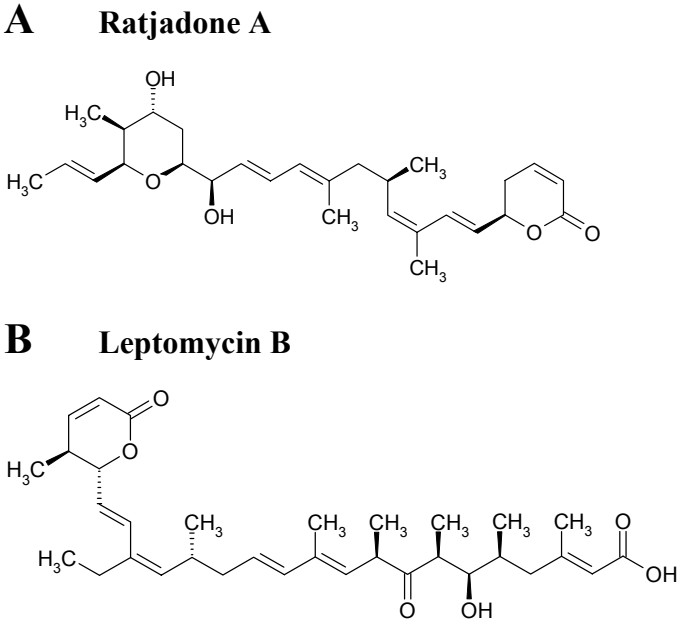


**Fig. 1.6** Chemical structures of the export inhibitor leptomycin B (LMB) and ratjadone A (RAT). Both polyketides contain a terminal α,β-unsaturated δ-lactone. The structures were prepared with ISIS/Draw 2.5 (MDL).

Nuclear export of proteins underlies the same restrictions as nuclear import. The majority of proteins of a molecular weight higher than ~40 kDa have to be actively exported from the nucleus in a carrier-assisted way (Görlich and Mattaj, 1996). The most prominent export receptor is exportin 1, also named CRM1 (Chromosome maintenance region 1, Adachi and Yanagida, 1989; Stade et al., 1997; Ossareh-Nazari et al., 2001). CRM1 recognises so-called nuclear export signals (NES) on its cargo proteins (Fischer et al., 1995; Wen et al., 1995b). Nuclear export signals are hydrophobic, predominantly leucine-rich stretches of about

10 amino acids that follow a loosely defined consensus sequence: L-x(2,3)-[LIVFM]-x(2,3)-L-x-[LI] (Henderson and Eleftheriou, 2000; la Cour et al., 2003). The study of nuclear protein export has greatly benefited from the discovery of the nuclear export inhibitor leptomycin B (LMB, see Fig. 1.6B). Originally isolated from *Streptomyces sp*. (Hamamoto et al., 1983a, b) as actinobacterial antibiotic with growth-inhibitory activity on yeast and filamentous fungi, the drug has been shown to specifically bind to and inactivate the nuclear export receptor CRM1 (Wolff et al., 1997; Kudo et al., 1997). Kudo and colleagues (1999) demonstrated that LMB covalently binds to a single sulfhydryl group of CRM1. The modification of $Cys^{528}$ of CRM1 from *S. pombe* and *H. sapiens* was shown to block association of CRM1 with the NES-containing cargo, thereby blocking CRM1-mediated export.

A separate chapter of this thesis (3.2) will be dedicated to the introduction and characterisation of ratjadone A as a new inhibitor of nuclear protein export and its use in the study of STAT nucleocytoplasmic transport. Ratjadone A was kindly provided by Dr. Markus Kalesse (Free University Berlin, now University of Hannover).

*Aim of the thesis*

The amino-terminal domain of the STAT proteins (N-domain) is a highly conserved, multi-functional protein domain. It has been implicated in such diverse processes as receptor association, oligomerisation, nuclear import and inactivation of STAT proteins, as well as co-factor recruitment. How a protein domain of only 135 amino acid residues can be engaged in so many different processes still remains a puzzling issue.

In this work a structure-function analysis will be performed in order to clarify the role of the STAT1 N-domain in nuclear accumulation of the transcription factor. Guided by the crystal structure, N-terminal deletion and substitution mutants of STAT1 will be constructed and analysed for their nuclear import behaviour. Since nuclear translocation and tyrosine dephosphorylation of STATs seem unequivocally linked to each other, the mutants will also be analysed for their dephosphorylation kinetics.

Moreover, a re-investigation of STAT1 arginine methylation was included in this work in order to understand the impact of this unusual post-translational modification of the N-domain on cytokine-induced gene expression.

In an additional part of this thesis, the myxobacterial cytotoxin ratjadone A will be characterised as an inhibitor of CRM1-mediated nuclear protein export. Ratjadone A will be used to analyse the subcellular localisation of STATs in resting cells.