REVIEW

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Mechanisms and consequences of Jak–STAT signaling in the immune system

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Kinases of the Jak ('Janus kinase') family and transcription factors (TFs) of the STAT ('signal transducer and activator of transcription') family constitute a rapid membrane-to-nucleus signaling module that affects every aspect of the mammalian immune system. Research on this paradigmatic pathway has experienced breakneck growth in the quarter century since its discovery and has yielded a stream of basic and clinical insights that have profoundly influenced modern understanding of human health and disease, exemplified by the bench-to-bedside success of Jak inhibitors ('jakinibs') and pathway-targeting drugs. Here we review recent advances in Jak–STAT biology, focusing on immune cell function, disease etiology and therapeutic intervention, as well as broader principles of gene regulation and signal-dependent TFs.

Discovery of the Jak–STAT pathway resulted from a logical progression of empirical findings related to the anti-viral activity of interferons and their ability to rapidly instruct gene expression in human cells[1]. Now known to operate downstream of >50 cytokines and growth factors, it is regarded as a central communication node for the immune system, as evinced by the dramatic immunological phenotypes seen in humans and mice bearing loss- or gain-of-function mutations of genes encoding Jak–STAT components. Also striking is the success of jakinibs, which now benefit thousands of patients with neoplastic or inflammatory pathologies and epitomize the clinical translation of basic immunology research. The history and fundamentals of Jak–STAT biology have been previously covered[2,3], so our aim is not to be comprehensive but, instead, to present current views of how Jak–STAT signaling works at cellular, molecular and genomic levels, focusing on immunological concerns and highlighting key practical and conceptual questions that will frame the second quarter century of Jak–STAT research.

Jaks and jakinibs

The mammalian Jaks (JAK1, JAK2, JAK3 and TYK2) were codified as essential mediators of cytokine and hormone-receptor signaling in the early 1990s[4]. Their clinical relevance was vividly illustrated with the discovery of JAK3-SCID ('severe combined immunodeficiency'), a human immunodeficiency syndrome caused by loss-of-function mutations in *JAK3* (refs. 5,6), and it was quickly confirmed by the extreme developmental and immunological phenotypes seen in Jak-deficient mice[4]. At that time, research in *Drosophila*[7] presaged another seminal finding: that gain-of-function mutations in Jak-encoding genes cause myeloproliferative disease[8–10]. Along with countless studies linking Jak–STAT to malignancies and inflammatory pathologies, these observations provided the impetus for Jak-targeting drugs and the rationale for applying them to both cancers and immune-system-mediated disease.

The first jakinib approved by the US Food and Drug Administration (FDA) was ruxolitinib. A potent inhibitor of both JAK1 and JAK2 is effective in the treatment of myeloproliferative neoplasms[11] and is now being tested for the treatment of various malignancies, including those not typically associated with underlying mutations in Jak-encoding genes, as well as a range of inflammatory disorders, including psoriasis and alopecia areata[12]. The second FDA-approved jakinib was tofacitinib, which blocks JAK1 and JAK3 (and, to a lesser extent, JAK2). It is approved for the treatment of rheumatoid arthritis[13] (six phase 3 studies have followed more than 6,000 patients for more than 8 years) and is currently the subject of more than a dozen clinical trials for the treatment of inflammatory pathologies, including juvenile arthritis, psoriasis, alopecia areata, ankylosing spondylitis, lupus and ulcerative colitis[14]. The third FDA-approved jakinib was oclacitinib, which has revolutionized the treatment of atopic dermatitis in dogs[13]. Building on those triumphs, second-generation jakinibs are now being developed[15]. Several of these claim improved Jak selectivity, although it bears noting a broader target range might contribute to the efficacy of the first-generation drugs[16]. Another emerging idea is to combine jakinibs with drugs that target other pathways, such as inhibitors of the kinase PI3K or receptor tyrosine kinases (RTKs), for the treatment of aggressive or refractory diseases[17–19].

Beyond their clinical applications, jakinibs have become powerful research tools. For example, two studies have used them to delineate biochemical events downstream of the interleukin 2 (IL-2) receptor in T cells; one showed that there are two separate waves of Jak activity[20], and the other showed that >80% of the IL-2-driven phospho-proteome (all phosphorylated proteins in a cell) is Jak dependent, a surprising finding given the various signaling pathways emanating from this receptor[21]. Other notable examples include studies using jakinibs to demonstrate that cytokines 'preferentially' affect

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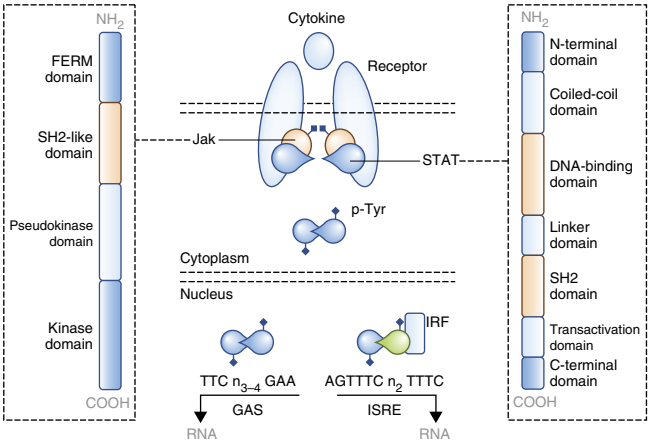

Figure 1 The canonical Jak–STAT pathway. Canonical Jak–STAT signaling begins with extracellular association between cytokines and their corresponding transmembrane receptors (top, middle). Receptor oligomerization then precipitates the trans-activation of Jaks that, in turn, phosphorylate the cytoplasmic tails of the receptors to create the requisite docking sites for STATs. This puts Jaks and STATs in spatial proximity and allows the former to mediate tyrosine-phosphorylation (p-Tyr) of the latter, which results in STAT dimerization, nuclear translocation, DNA binding and, ultimately, modulation of gene transcription. All STATs can engage the GAS motif, except STAT2, which participates in a trimeric complex (STAT1–STAT2–IRF9) that engages the ISRE. Mammalian Jaks are composed of four domains (far left): the FERM domain, which mediates interaction with upstream receptors and promotes kinase function; the SH2-like domain, which also mediates interaction with upstream receptors; the pseudokinase domain, which limits unwarranted kinase activity; and the kinase domain, which contains the tyrosine residues necessary for trans-activation and the catalytic elements necessary for the tyrosine-phosphorylation of receptors, Jaks and STATs. Mammalian STATs are composed of seven domains (far right): the N-terminal domain, which is involved in protein-protein interactions; the coiled-coil domain, which is also involved in protein-protein interactions and contains nuclear-localization signals; the DNA-binding domain, which directly interfaces with DNA and contains nuclear import–export signals; the linker domain, which is structurally important and promotes transcriptional activity; the SH2 domain, which mediates dimerization and interaction with upstream receptors; the transactivation domain, which contains the phosphorylated tyrosine residues necessary for canonical signaling; and the C-terminal domain, which contains phosphorylated serine residues that support both canonical functions and non-canonical functions.

genes bearing super-enhancers (dense enhancer clusters that appear at tightly regulated loci[22]), to assess the effect of homeostatic cytokines in immune cells[23], to catalog transcriptomic effects of tonic and acute interferon signaling[23,24] and to probe molecular mechanisms for drug resistance in certain cancers[25–27].

Given the success of jakinibs, there is keen interest in defining the molecular events that lead to the activation of Jaks. Early work identified four key protein domains[4] (**Fig. 1**), and biophysical analyses have since probed each step of the signaling cascade: the interaction between upstream receptors and their ligands[28], the dynamics of receptor oligomerization[29], the interaction between Jaks and upstream receptors[30–34] and the conformational changes in upstream receptors that enable or disable the enzymatic activity of Jaks[35]. In addition, several studies have focused on the Jaks themselves and, specifically, their pseudokinase domain, the region most commonly affected by oncogenic mutations. These have revealed previously unrecognized enzymatic activity[36,37] and have provided detailed structural understanding of how that domain interacts with and thereby regulates the kinase domain and, in turn, how genetic lesions unleash Jak hyperactivity[26,38,39].

STATs as transcription factors: a genome-wide view

Genes encoding the seven mammalian STATs (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6) are grouped in three genomic clusters that are testament to ancestral gene duplications[40]. Accordingly, comparative genetics bear witness to both common origins and stepwise evolution. Certain genetic modules are conserved across the entire family and, in turn, all members share seven characteristic protein domains[3] (**Fig. 1**). In addition, crystallographic studies have revealed a common logic for how they interact with upstream receptors, with each other (i.e., dimerization and tetramerization) and with DNA[3,41–43]. Some family members are clearly more closely related than others, as with the extreme example of STAT5A and STAT5B, which were formed during the most recent duplication and are >90% similar at the protein level, but there is also emerging evidence that key structural distinctions enable member-specific activity, as has been demonstrated for STAT6 (ref. 44).

STATs are classic TFs, in that they directly engage DNA regulatory elements (DREs) and thereby control the transcription of associated genes. Initially, low-throughput, candidate-driven strategies were used to identify STAT-regulated DREs, typically within 1 kilobase of genes known to be influenced by upstream cytokines. However, due to the labor-intensive nature of this work-flow, only a handful were discovered[45]. The advent of next-generation sequencing has since removed that barrier. Through the coupling of massively parallel DNA sequencing with chromatin immunoprecipitation (ChIP-seq), it is now possible to capture thousands of STAT-binding sites without a priori predictions. These unbiased maps can then be integrated with loss- or gain-of-function transcriptomics to catalog target genes that are subject to both proximal STAT occupancy and STAT-dependent transcriptional regulation (i.e., STAT target genes). This approach was first applied to STAT1 in HeLa human cervical cancer cells[46] and has since been used to assess all family members in primary immune cells (**Supplementary Table 1**). Such work has made it clear that beyond proximal DREs (i.e., promoters), STATs often localize far from protein-encoding genes. These distal binding events can be segregated into three general categories—enhancers, epigenetic hotspots, and non-coding loci (i.e., microRNAs and long intergenic noncoding RNAs)—although it must be acknowledged that these designations are neither exclusive nor exhaustive and that not all binding events can be so classified (**Fig. 2**).

Enhancers, loosely defined as regions of DNA that promote gene expression via protein activators (i.e., TFs), are quintessential DREs. Despite growing evidence for inter-chromosomal effects, they generally act in *cis*, sometimes proximal to target genes but more often distal, operating as far as 1 megabase away. With next-generation sequencing, it is now possible to comprehensively map enhancers on the basis of characteristics epigenetic (for example, histone acetylation) and TF-binding patterns (for example, p300 or the Mediator complex) or by the detection of physical interactions with target genes[47]. Those methodologies have been applied to immune cells to demonstrate that STATs typically congregate at enhancers and, in fact, are major architects of active enhancer landscapes[48–50] (**Fig. 2**). Such studies have confirmed the long-standing idea that STATs control enhancer activity at emblematic, lineage-defining loci, such as *Ifng* and *Il4–Il13* in T cells[51], and have expanded their purview genome wide. They have also revealed that STAT-driven remodeling is often upstream of lineage-defining TFs, such as T-BET, GATA3 and PU.1, and have thereby established that STATs and, more generally, signal-dependent TFs can be primary arbiters of enhancer activity[48,49].

An important application of ChIP-seq is the correlation of TF distribution with epigenetic modifications. Studies of immune cells

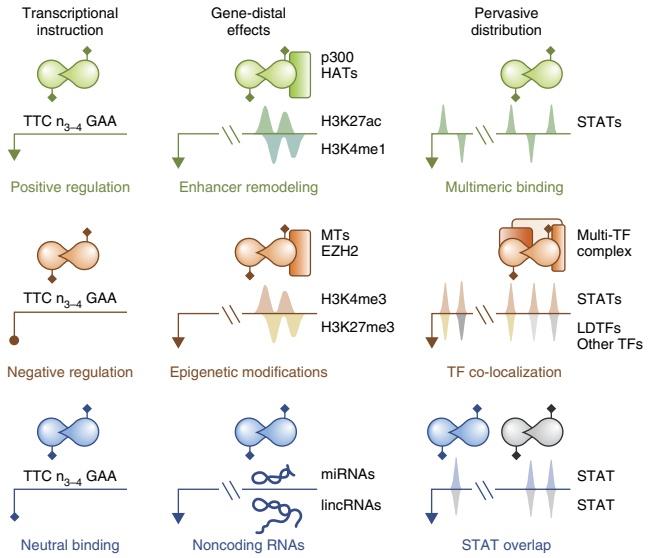

Figure 2 STATs as TFs. STATs are classic TFs in that they engage DREs bearing a particular sequence motif and thereby instruct the transcription of nearby genes. However, many gene-proximal binding sites are not clearly linked to gene transcription (i.e., neutral binding). STATs also engage distal DREs and control enhancer activity and/or epigenetic status of associated genes, sometimes through physical interaction with histone acetyltransferases (HATs), such as p300, or methyl transferases (MTs), such as EZH2, or directly instruct distal non-coding loci (i.e., miRNAs and long intergenic noncoding RNAs (lincRNAs)). STAT-regulated DREs are often studded with multiple binding sites that tend to attract more than one family member (i.e., STAT overlap) and co-localize with those of other TFs, sometimes reflective of the inclusion of STATs within multi-TF complexes. H3K27ac, histone H3 acetylated at Lys27; H3K4me1, histone H3 mono-methylated at Lys4; LDTF, lineage-defining transcription factor.

have established that STATs can promote the deposition of permissive epigenetic marks (for example, tri-methylation of histone H3 at Lys4 (H3K4me3)) or repressive epigenetic marks (for example, tri-methylation of histone H3 at Lys27 (H3K27me3)) at loci that exhibit both STAT binding and STAT-dependent transcriptional regulation (**Fig. 2**). This effect has been clearly demonstrated for STAT3, STAT4 and STAT6 in T cells[52,53] and for STAT1 in macrophages[50,54]. STAT5-driven epigenetic modifications have not been comprehensively mapped in immune cells, but studies of mammary epithelium have shown that it is also capable of promoting H3K4me3 and histone acetylation at bound, transcriptionally responsive loci[55,56]. Exactly how STATs elicit epigenetic remodeling, whether by transcriptional regulation and/or by recruitment of the underlying machinery, remains uncertain. There is evidence for both; transcriptomics studies suggest that STATs control the expression of various chromatin modifiers, and physical interactions between STATs and CBP (p300), which mediates histone acetylation, or EZH2, which mediates deposition of H3K27me3, have been demonstrated[57,58].

Cross-referencing of ChIP-seq data for STATs with that for other TFs has revealed that they often participate in multi-factorial networks that assemble at transcriptionally relevant DREs, such as promoters or enhancers (**Fig. 2**). The idea that STATs physically interact with other TFs has long been appreciated; venerable examples include STAT1-STAT2 and IRF9 (ref. 59), STAT3 and JUN[60], and STAT5 and GR[61]. The idea that STATs co-localize and act together with other TFs at certain DREs is also well documented. For example, in macrophages, STAT1 and NF-κB bind adjacent sites and work in concert to control transcription of the gene encoding nitric oxide synthase[62].

With the advent of next-generation sequencing, this principle has now been extended genome wide. Salient examples of this have been recognized in stem cells, where STAT3 participates in a network that includes OCT4, SOC2, and NANOG[63]; in mammary epithelium, where STAT5 participates in a network that includes GR, ELF5 and NFIB[56]; in T cells, where STAT3 participates in a network that includes IRF4, BATF and RORγt[64,65]; in dendritic cells (DCs), where STAT1 participates in a network that includes IRF1, IRF8 and NF-κB[66]; and in macrophages, where STAT1 participates in a network that includes IRF1, IRF8 and PU.1 (ref. 67). Another emerging theme is that STATs often co-localize with lineage-defining TFs, as in T cells, where they co-localize with T-BET, RORγt, FOXP3 and GATA-3 (refs. 48,64,68), suggestive of an intimate relationship. Whether STATs are pioneers or just essential components in these multi-molecular networks is a key open question. The answer is surely context dependent and probably varies from network to network or even from gene to gene, but one study has shown that IRF4 necessarily precedes STAT3 at many target genes in T cells[69].

It is now common knowledge, but the pervasive nature of STAT binding was initially surprising to many in the field. It had long been appreciated that certain loci bear multiple interferon-γ (IFN-γ)-activated sequence (GAS) elements, as in the classic example of *Ifng* (which encodes IFN-γ)[70], but ChIP-seq has since revealed that hundreds of genes are decorated with five or more islands of proximal STAT binding in immune cells. Closely spaced 'tandem' sites are known to act together for optimal transcription, as with STAT5-driven expression of *IL2RA* (which encodes the cytokine receptor subunit IL-2Rα)[71], but it remains unclear how more distantly spaced sites influence each other. One study has addressed this issue using a genetic approach and has revealed a hierarchy between non-tandem STAT5 binding in controlling the transcription of *Wap* (which encodes whey acidic protein) in mammary epithelium[72]. However, it is unknown whether similar logic applies to other genes or in other cell types. Thus, a pressing challenge is to devise a set of rules that can be used to predict relative importance among non-tandem sites. Do those closest to the transcription start site generally have a greater effect? Does the amount of STAT binding (i.e., ChIP-seq peak amplitude) correlate with transcriptional relevance? Do all sites tend to be occupied at the same time and/or by the same STAT family member? These are just a few of the questions to be addressed.

Another unexpected finding is the extensive co-localization of different STATs. This trend is plainly evident in helper T cells[73], where multiple STATs often bind the same genomic locales and have analogous effects on the transcription of nearby genes. Among the many immunologically relevant examples are *Il2ra*, which is positively regulated by STAT3, STAT4 or STAT5 (refs. 74–76); *Il2* (encoding IL-2), which is negatively regulated by STAT4, STAT5 or STAT6 (ref. 77), and *Prdm1* (encoding the transcriptional repressor Blimp-1), which is positively regulated by STAT3, STAT4 or STAT5 (refs. 78–80). It has also become apparent that many STAT-binding sites do not contain GAS motifs, which suggests either degeneracy in the recognition code or alternative targeting strategies, such as tethering to other TFs[81], and that many (if not most) GAS-bearing, proximal binding sites are not obviously linked to transcriptional effects. With regard to the latter issue, it is impossible to exhaust all possible scenarios in which a given gene might be subject to STAT-dependent regulation, but it does seem unlikely that all gene-proximal binding events are transcriptionally pertinent. As noted by Bertrand Russell, "nobody can prove that there is not between the Earth and Mars a china teapot revolving in an elliptical orbit, but nobody thinks this sufficiently likely to be taken into account in practice"[82].

The STAT specificity paradox

All members of the STAT family bind GAS elements (**Fig. 1**). The nucleotides between and around the core palindromic motif impart some level of selectivity (certain STATs bind certain GAS variants better than others), but all family members can engage each other's 'preferred' sites[45]. The two notable exceptions are STAT2, which associates with STAT1 and IRF9 in a trimeric complex (ISGF3) that binds a distinct sequence motif (i.e., the ISRE ('interferon-stimulated response element'))[2], and STAT6, which 'prefers' four spacer nucleotides (instead of the canonical three) between palindromes[83,84], although both are still capable of accessing conventional GAS sites. Nevertheless, each STAT does have unique functions at cellular and organismal levels, so it is clear that specificity can be achieved despite this molecular redundancy, perhaps through a select group of genes whose expression is solely (or at least dominantly) regulated by one (or few) STAT(s). The most straightforward explanation is that subtle differences in GAS-variant affinity allow each family member to dominate a particular set of DREs that, in turn, control a particular set of genes (i.e., a transcriptional signature) upstream of a particular set of cellular functions. Epigenetics probably factor in this hierarchy; each STAT may differ in how it interacts with DNA topologies or histone modifications, which might explain why experiments using synthetic oligonucleotides tend to conclude that all STATs have similar binding properties, while those involving native chromatin tend to champion member-specific abilities. This model accounts for the fact that some genes are particularly sensitive to certain STATs, as well as the long-standing observation that in some settings, the loss of one STAT results in the compensatory deployment of other family members. For example, STAT1-activating cytokines display enhanced STAT3 responses in the absence of STAT1 (and vice versa), and STAT5-activating cytokines can use STAT6 in the absence of STAT5 (refs. 85–87). Notably, these findings illustrate redundancy in two cellular compartments: the cytoplasm, where one receptor must activate multiple STATs; and the nucleus, where different STATs often engage the same target genes. Another point to consider is that specificity is lineage dependent; the target repertoire for a given STAT varies from one cell type (or state) to another, due in part to epigenetic constraints that allow or disallow access to distinct panels of genes.

STATs physically associate with other TFs, so it stands to reason that heterologous protein interactions contribute to functional specificity, as with the classic example of ISGF3. Thus, while multiple STATs may engage a given DRE, only certain family members may participate in the multi-molecular complexes necessary for transcriptional induction or repression. This is in line with the emerging concept of STAT competition, whereby two family members compete for access to the same genomic locales and mediate distinct outcomes. In that scenario, it is inferred that one STAT empowers the transcriptional machinery while the other restrains it by enlisting transcriptional inhibitors or by steric hindrance, because it simply occludes the space that would be occupied by pro-active STATs. Genome-wide competition was first described for STAT3 and STAT5 in the context of helper-T- cell-subset specification[88] and has since been evoked in this and other settings in which they display opposing functions[56,89–92]. Whether other STATs exhibit this type of broad antagonism remains unclear, but one study has demonstrated that although STAT1 and STAT3 bind thousands of common sites in helper T cells and have opposing effects on subset specification, head-to-head competition is restricted to a small set of genes that happens to include those encoding key elements of the T_H1-subset and T_H17-subset differentiation programs[93].

Most (if not all) cytokines activate multiple STATs. For example, IL-12 is the prototypical STAT4 stimulus but also activates STAT3 (ref. 94), IL-4 is the prototypical STAT6 stimulus but also activates STAT5 (ref. 95), and type I interferons are prototypical stimuli for STAT1 and STAT2 but also activate STAT4 (ref. 96). In some cases, cytokines enlist multiple STATs with comparable efficiency, as with IL-27, which is a strong activator of both STAT1 and STAT3 (ref. 97), while for others there is clear disparity, as with IFN-γ, which elicits a strong STAT1 response coupled to a weaker STAT3 response[86]. Heterogeneous STAT signaling is now accepted as biological truth, but few studies have systematically addressed its role in defining specificity; graded combinations of auxiliary STATs might distinguish cytokines that 'look' similar in terms of primary STATs but have different functions. IL-6 and IL-10 are prime examples of this. Each employs STAT3 as their principal signaling moiety, but their downstream cellular effects are clearly distinct, as evinced by their respective pro-inflammatory activities and anti-inflammatory activities in myeloid cells. Qualitative differences in the duration and/or intensity of STAT3 signaling are one explanation for this disparity, but given that both employ auxiliary STATs, heterogeneous signaling might also contribute[98]. Likewise, IL-6 and IL-27 both activate STAT3 and share a STAT3-driven transcriptional signature in helper T cells. However, each has distinct functions and unique sets of target genes that are determined, at least in part, by variable usage of STAT1 (ref. 93), a finding echoed by studies demonstrating that a portion of the IL-21-driven transcriptome is dependent on STAT1 rather than STAT3, its primary signaling agent[99].

'Fine tuning' Jak–STAT signaling

Every step of the Jak–STAT pathway is tightly monitored (**Fig. 3**). One key regulatory module involves a long (and growing) list of phosphatases, including SHP1, SHP2, CD45, PTPRD, PTPRT, TCPTP, PTP1B and DUSP2 (refs. 100–102), which hydrolyze phosphorylated tyrosine residues on Jaks, STATs and upstream receptors. Another involves proteins of the PIAS ('protein inhibitor of activated STAT') family. These suppress STAT-driven gene transcription through a variety of mechanisms that typically involve physical interactions within the nucleus and affect a range of immunological processes, as exemplified by PIAS1, an important regulator of STAT1 in macrophages[103] and of STAT5 in regulatory T cells (T_{reg} cells)[104]. A third prominent module involves proteins of the SOCS ('suppressor of cytokine signaling') family. In mammals, four members of this family (CISH, SOCS1, SOCS2 and SOCS3) are intimately linked with the Jak–STAT pathway. They act as negative feedback circuits (each is transcriptionally induced by the STAT they are meant to restrain), but there is also promiscuity: one STAT might induce multiple SOCSs and, in turn, one SOCS might regulate multiple STATs[105]. Like STATs, SOCS proteins contain SH2 domains that enable receptor binding and put them in proximity to Jaks, whose kinase domain they directly suppress[106]. By controlling the intensity and/or duration of Jak activity, SOCS proteins dictate both quantitative aspects and qualitative aspects of cytokine signaling; the latter is clearly illustrated by studies showing that IL-6 switches from a STAT3-driven transcriptional signature to a STAT1-driven transcriptional signature in the absence of SOCS3 (refs. 107,108). Consequently, they have profound effect on immune cells, as evinced by the lethal inflammatory phenotypes seen in SOCS1-deficient mice[105] and studies describing the role of CISH in pro-allergic T cell responses[109], the role of SOCS1 in T cell development and T_{reg} cell function[110,111], the role of SOCS2 and SOCS3 in macrophage differentiation[112], and the role of SOCS3 in the function of natural killer (NK) cells and $CD8^+$ T cells[113,114].

A common assumption is that the cytoplasmic pool of latent STATs is kept at saturating density. In reality, STATs are a limiting resource,

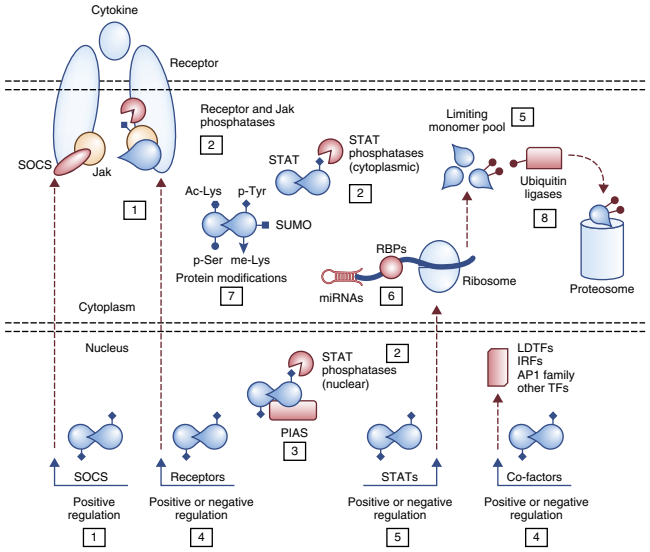

Figure 3 'Fine-tuning' Jak–STAT signaling. The Jak–STAT pathway is subject to multiple levels of regulation. STATs induce the transcription of genes encoding SOCS proteins that, in turn, limit STAT activity via inhibitory interactions with receptors and Jaks (1). Phosphatases hydrolyze key tyrosine residues necessary for signal transduction by receptors, Jaks and STATs (2). PIAS proteins suppress STAT-driven gene transcription (3). STATs control the transcription of genes encoding receptors and TFs that influence STAT activity (4), as well as genes encoding the STATs themselves (5). MicroRNAs and RNA-binding proteins (RBPs) influence the half-life and translation of STAT mRNA (6). Post-translational modifications, including serine phosphorylation, lysine acetylation, lysine methylation and sumoylation, promote or suppress STAT activity (7). Ubiquitin ligases drive the proteasomal degradation of STATs (8). AP1, a family of TFs.

and even subtle changes in cellular concentration can have important consequences. For example, oscillating levels of STAT1 and STAT4 dictate responsiveness to upstream cytokines in $CD8^+$ T cells and NK cells[115,116], and changes in the availability of STAT5 or STAT4 can profoundly influence T cell function; the former affects both follicular-helper-T-cell specification and T_{reg}-cells specification[117], and the latter affects T_H2-lineage specification[118]. It has long been appreciated that the transcription of STAT-encoding genes is labile and is subject to a range of upstream stimuli, including STATs themselves; certain family members can induce transcription of their own genes[119]. In addition, most STAT-encoding loci bear super-enhancers, which suggests precise supervision[22], as highlighted by work describing a STAT5-driven super-enhancer that controls the *Stat5a–Stat5b* locus[120]. Further evidence that STAT protein concentrations are tightly monitored comes from the field of post-transcriptional regulation. Numerous microRNAs influence the translation and/or degradation of STAT mRNAs and, given the appearance of AU-rich elements in these transcripts, RNA-binding proteins are probably involved[121]. In addition, it is well established that the ubiquitin–proteasome system controls the turnover of STAT proteins in immune cells[122], and various E3 ligases have been implicated, including SLIM, PDLIM2 and GRAIL[123–125].

Jak–STAT signaling is typically presented as a simple, linear pathway with one key biochemical trigger (i.e., Jak-driven tyrosine phosphorylation) and few molecular components. However, less-well-understood non-canonical tangents can also affect cytokine signaling and immune cell function[126]. For example, the canonical model posits that STATs act mostly as homodimers, but they are also known to form heterodimers and higher order tetramers. In fact, a founding doctrine in the field is that type I and type II interferons differ because the former employs STAT1–STAT2 heterodimers while the latter employs STAT1–STAT1 homodimers[2]. Numerous other examples have since been reported, including STAT1–STAT3 and STAT1–STAT4 heterodimers, but their functional relevance remains largely unexplored[127]. The importance of tetramers is also underappreciated. It is well established that mutant STATs capable of forming dimers but not tetramers exhibit decreased binding and transcriptional activity at tandem GAS-bearing genes[71,128], and broader *in vivo* consequences have begun to emerge. For example, tetramerization of STAT1 is required for the anti-microbial activities of type II interferons but not for the anti-viral activities of type I interferons[129], and tetramerization of STAT5 is important for NK cell homeostasis, T_{reg} cell function and cytotoxic T cell proliferation[130].

Tyrosine phosphorylation is considered the *sine qua non* of STAT function. However, there is a growing body of evidence suggesting that 'unphosphorylated' STATs also have important cellular effects. *In vitro* experiments have identified several immunologically relevant functions, such as the ability to prolong interferon- or IL-6-driven gene transcription[131,132], and *in vivo* correlates have started to emerge, with studies demonstrating that unphosphorylated STAT3 and unphosphorylated STAT5 are critical for stem cell pluripotency and stem cell hematopoiesis, respectively[133,134]. There is also growing awareness of other chemical modifications that can influence STAT activity in immune cells. Notable findings involve serine-phosphorylation (p-Ser) of STAT1 in NK cells[135], p-Ser and sumoylation of STAT1 in interferon-driven gene transcription[136,137], p-Ser of STAT3 in mitochondrial respiration and cancer[138,139], p-Ser of STAT3 or STAT5 in hematopoietic transformation[140,141], methylation of STAT3 in IL-6-driven gene transcription[142], and acetylation of STAT5 in T_{reg} cell function[143].

Immune cells are often exposed to a rich tapestry of extracellular stimuli. Thus, it is not surprising that parallel signaling pathways often influence Jak–STAT activity. For example, the relationship between STAT1 downstream of interferons and NF-κB downstream of Toll-like receptors or the cytokine TNF has long been appreciated (the two pathways can promote or 'prime' each other[144,145]), and studies have identified important interactions between STATs and receptors bearing immunoreceptor tyrosine-based activation motifs, which modulate STAT1 (ref. 146), and the TRAF family of receptors, which modulate STAT3 and STAT5 (refs. 147,148). It is also not surprising, given the importance of Jak–STAT for resistance to infection, that pathogens have evolved a range of evasion strategies, some of which co-opt or mimic the regulatory mechanisms described above. Many involve STAT1, well known for its anti-viral and anti-microbial properties, including classic examples discovered in poxviruses, which encode interferon-receptor homologs[149]; vaccinia viruses, which encode phosphatases[150]; and Epstein-Barr virus, which encodes a protein that subverts multiple components of the interferon–STAT1 axis[151]. As with viruses, prokaryotic and eukaryotic pathogens can also manipulate Jak–STAT signaling in infected cells, as epitomized by *Toxoplasma gondii,* an intracellular protozoan that secretes proteins that promote the activation of STAT3 and STAT6 (refs. 152,153) or obstruct STAT1-driven gene transcription[154,155].

Lessons from human and mouse genetics

Following on JAK3-SCID, numerous germline gain- or loss-of-function mutations in genes encoding Jak–STAT components have been identified[156] (**Table 1**). Immunodeficiency and susceptibility to infection are the most common attendant phenotypes, which highlights the cardinal role of Jak–STAT signaling in the development

and/or function of immune cells. However, anti-inflammatory properties are also evident, as in patients with loss-of-function *STAT5B* mutations, who develop autoimmune complications due in part to T_{reg} cell defects[157], and patients with gain-of-function *STAT1* mutations, who suffer from recurrent *Candida* infections due to antagonism of STAT3-driven anti-fungal responses[158]. Beyond rare monogenic diseases, genome-wide association studies, in which disease phenotypes are linked to single-nucleotide polymorphisms in affected populations, have linked Jak–STAT to a range of common maladies, including rheumatoid arthritis, psoriasis and asthma (**Table 1**). In addition, the list of somatic mutations linked to oncogenic Jak–STAT hyperactivity is ever growing. Aside from the classic *Jak2*V617F allele[8–10], several gain-of-function variants of Jaks and upstream receptors have been linked to malignancy[159], as have mutations and fusions of STAT-encoding genes[160–169] (**Table 1**). However, it should be noted that the latter are less common, and it is generally believed that oncogenic STAT hyperactivity is secondary to upstream events, such as increased availability or receptiveness to upstream cytokines or gain-of-function mutations of genes encoding receptors or Jaks.

In general, knockout mice lacking Jak–STAT components recapitulate the phenotypes of humans bearing loss-of-function mutations. Thus, despite a few notable exceptions (for example, JAK3-deficient mice lack B cells (unlike patients with JAK3-SCID[4]), and STAT5B-deficient mice exhibit distinct (and less severe) autoimmune manifestations[117]), they have enabled scores of important, often unexpected, discoveries related to human immunology. STAT1-knockout mice are a prime example. They are predictably susceptible to intracellular pathogens, which has made them invaluable for the study of anti-microbial immunity[158], and are surprisingly hyper-sensitive to certain inflammatory pathologies[170,171], which has prompted the study of immunosuppressive

Table 1 Immunological features of mutations of genes encoding Jak–STAT components in humans

Component	Germline loss-of-function disease	pLI	Germline gain-of-function disease	GWAS diseases	Hematopoietic and lymphoid malignancies	Mutation frequency
JAK1	Bacterial infections (intracellular)	100%	NR	Diabetic kidney disease	Acute lymphoblastic leukemia; acute myeloid leukemia	1.2%
JAK2	NR	97%	Thrombocythemia	Myeloproliferative neoplasms; inflammatory bowel disease; pediatric autoimmune diseases	Myeloproliferative neoplasms; acute lymphoblastic leukemia; acute myeloid leukemia; chronic myelogenous leukemia; essential thrombocythemia	38.7%
JAK3	SCID	100%	NR	NR	Myeloproliferative neoplasms; acute lymphoblastic leukemia; myelomonocytic leukemia; NK lymphoma-leukemia; T cell lymphoma-leukemia	2.2%
TYK2	Bacterial infections (intracellular); viral infections	0%	NR	Inflammatory bowel disease; systemic lupus erythematosus; multiple sclerosis; psoriasis; rheumatoid arthritis; primary biliary cirrhosis; pediatric autoimmune diseases	NR	0.5%
STAT1	Bacterial infections (intracellular); viral infections	100%	Mucocutaneous candidiasis; bacterial infections (extracellular); bacterial infections (intracellular); viral infections; autoimmune manifestations	Inflammatory bowel disease; systemic lupus erythematosus; primary biliary cirrhosis	T cell lymphoma-leukemia	0.3%
STAT2	Viral infections	4%	NR	Psoriasis	NR	0.1%
STAT3	Hyper-IgE syndrome; mucocutaneous candidiasis; bacterial infections (extracellular); viral infections	100%	Bacterial infections (intracellular); viral infections; autoimmune manifestations; immunodeficiency	Inflammatory bowel disease; multiple sclerosis; atopic dermatitis; psoriasis	B cell lymphoma; NK cell lymphoma-leukemia; T cell lymphoma-leukemia; myelodysplastic syndrome	5.0%
STAT4	NR	99%	NR	Inflammatory bowel disease; systemic lupus erythematosus; rheumatoid arthritis; Behcet's disease; primary biliary cirrhosis; Sjögren's syndrome; systemic sclerosis	NR	0.1%
STAT5A	NR	100%	NR	NR	NR	0.1%
STAT5B	Bacterial infections (intracellular); viral infections; chronic pulmonary inflammation; autoimmune manifestations; immunodeficiency	100%	NR	NR	Acute lymphoblastic leukemia; chronic lymphocytic leukemia; T cell lymphoma-leukemia; large granular lymphocytic leukemia	1.6%
STAT6	NR	10%	NR	Atopy; asthma; eosinophilic esophagitis	B cell lymphoma; follicular lymphoma	1.2%

Common phenotypes associated with loss- or gain-of-function mutations compiled from the Online Mendelian Inheritance in Man database (https://www.omim.org/). Predicted loss-of-function intolerance (pLI) indicates the probability that loss-of-function variants will be tolerated (100% indicates extreme intolerance). Data were obtained from the Exac database (http://exac.broadinstitute.org). Diseases associated with single-nucleotide polymorphisms were compiled from the NHGRI-EBI Catalog of Published Genome-Wide Association Studies (https://www.ebi.ac.uk/gwas). Hematopoietic and lymphoid malignancies associated with somatic missense mutations were compiled from the Catalogue of Somatic Mutations in Cancer database (http://cancer.sanger.ac.uk/cosmic) . The frequency of non-synonymous mutations within all sequenced samples is presented here. NR, not reported; GWAS, genome-wide association study.

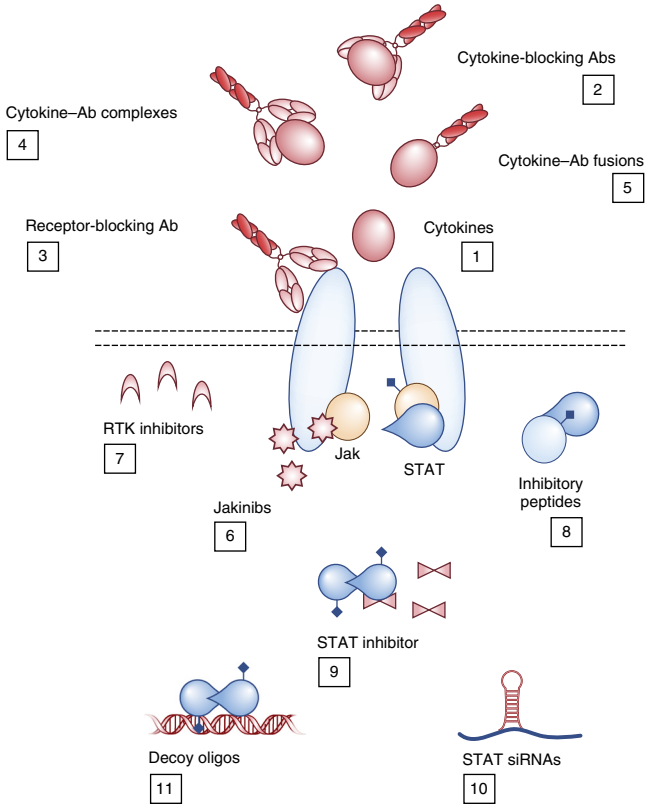

Figure 4 Therapeutic targeting of the Jak–STAT pathway. Numerous strategies have been developed for clinical manipulation of the Jak/STAT pathway. Those currently approved or undergoing clinical trials in the USA include recombinant and engineered cytokines (1), cytokine-blocking antibodies (Abs) (2), receptor-blocking antibodies (3), agonist cytokine–antibody complexes (4), agonist cytokine–antibody fusions (5), small-molecule Jak inhibitors (Jakinibs) (6), small-molecule RTK inhibitors (7), STAT-binding inhibitory peptides (8), small-molecule STAT inhibitors (9), STAT-targeting small interfering RNAs (siRNAs) (10) and STAT-binding decoy oligonucleotides (oligos) (11).

properties that are not readily apparent in STAT1-deficient humans. The ability to delete genes encoding components of the Jak–STAT pathway within defined cell types (or cell states) through the use of Cre-Lox recombination (i.e., conditional knockouts) has also been instrumental. The necessity and power of this approach is exemplified by STAT3. Germline deletion of the gene encoding STAT3 is lethal to embryos[172], so conditional knockout has been the only way to study its many pro- and anti-inflammatory properties, including its role in $CD4^+$ T cell differentiation[173–175], $CD8^+$ T cell memory[176], mucosal immunity and tissue repair[177], DC-lineage specification[178], DC-mediated immunotolerance[179] and myeloid-cell-mediated immunotolerance[180]. A similar story can be told for STAT5. Mice lacking both STAT5A and STAT5B exhibit a multitude of immunological defects and die soon after birth[181]. Thus, lineage-restricted deletion of STAT5 has been key for defining its *in vivo* functions, including its role in $CD4^+$ T cell differentiation[78,182–185], NK-cell-mediated immunosurveillance[186], $CD8^+$ T cell memory[187], DC-lineage specification[188] and DC-driven type 2 inflammation[189]. In contrast, lineage-restricted deletion of Jaks has not been widely adopted despite the severe developmental and immunological defects observed in knockout mice.

Unlike knockout mice, knock-in mice expressing mutant Jak–STAT proteins provide valuable structure–function information. They are also better approximations of the genetic aberrations found in humans, which tend to generate hypo- or hypermorphic proteins. Accordingly, knock-in mice bearing a *Jak2* allele commonly associated with myeloproliferative disorders[190] or a *Stat3* allele commonly associated with hyper-IgE syndrome[191] faithfully recapitulate immunological aspects of the corresponding human syndromes. Mutations inspired by basic science rather than by clinical observations have also been informative, such as mice bearing cytokine receptors with diminished or enhanced STAT binding[192,193] and mice bearing mutant STATs that cannot undergo p-Ser or tetramerization[129,130,135,194]. Going forward, genome-editing technologies (for example, CRISPR and TALEN) will facilitate the engineering of knock-in (and knockout) mice, which will allow researchers to further probe both protein-encoding regions of the genome non-coding regions of the genome, as has been done for the STAT5-dependent DREs that control the *Wap* locus in mammary epithelium[72]. They can also be applied to primary immune cells *in vitro* to bypass the ethical and financial barriers associated with transgenic animals and expand the number of genetic perturbations that can be tested, from one (or few) to potentially millions[195,196].

Therapeutic targeting of the Jak–STAT pathway

Given the mountains of data linking Jak–STAT to health and disease, it is not surprising that this pathway has become an eminent drug target (**Fig. 4**). One well-established approach is the manipulation of upstream activators. Recombinant cytokines have long been used in clinical settings; venerable examples include type I interferons for the treatment of hepatitis C and multiple sclerosis, and IL-2 for the treatment of renal carcinoma[197], and many new applications are currently being tested, such as a low dose of IL-2 for malignancies and autoimmune diseases; IL-12 and IL-15 for metastatic cancers; and IL-7 for lymphopenia[198]. In addition, engineered cytokines, agonist cytokine–antibody complexes, and agonist cytokine–antibody fusions have all shown promising results in animal models and are being adapted for clinical use[199,200]. On the other end of the spectrum, cytokine- and receptor-blocking antibodies have also been gaining traction. Several are FDA approved, including antibody to IL-2Rα (anti-IL-2Rα), anti-IL-5, anti-IL-6, anti-IL-6R, anti-IL-12 and anti-IL-23, and many others await licensing, including anti-IFN-α, anti-IL-4, anti-IL-4R, anti-IL-5R, anti-IL-6R, anti-IL-9 and anti-IL-13 (refs. 197,201). It can also be said that any agent that affects the availability of and/or responsiveness to STAT-activating cytokines is a *de facto* Jak–STAT modulator. For example, it is certain that one reason for the efficacy of immunotherapies such as checkpoint inhibitors or chimeric-antigen-receptor T cell therapies is that they elicit tumoricidal STAT-activating cytokines. The same reasoning applies to commonly used immunosuppressive drugs such as corticosteroids, cyclosporine or methotrexate; their efficacy must be due at least in part to effects on the production and/or activity of STAT-activating cytokines.

As discussed above, small-molecule inhibitors of Jaks (i.e., jakinibs) have proven effective for the treatment of myeloproliferative neoplasms and rheumatoid arthritis and are currently under trial for the treatment of various malignancies and inflammatory diseases. Small-molecule inhibitors of RTKs, a class of growth-factor and hormone receptors whose genes are prone to gain-of-function mutations associated with STAT-driven malignancy, are also being tested for the treatment of various cancers and inflammatory pathologies[202]. Unlike Jaks and RTKs, whose kinase domains present a clear pharmacological target, STATs do not have intrinsic catalytic activity and thus are a more challenging objective. Oligonucleotide-based STAT inhibitors, which presumably sequester the STATs away from the genome, are the most well-developed approach[203], and small molecules, inhibitory peptides and small interfering RNAs that target

STATs are also undergoing clinical trials[204,205]. Finally, STAT activity can be affected by drugs that are not necessarily pathway specific, such as pimozide, resveratrol, curcumin and platinum-based drugs, although it is unclear whether this is due to direct interaction with STATs or indirect mechanisms[204].

Closing statement

The Jak–STAT pathway is a paradigm of receptor-mediated signal transduction and a shining example of the clinical translation of basic immunology. As the field strides into its second quarter-century, technological advances will continue to facilitate ever deeper understanding of the pathway, including the cell-intrinsic and cell-extrinsic factors that govern Jak–STAT activity, molecular and genomic mechanisms underlying Jak–STAT–driven epigenetic and transcriptional effects, and the genetic causes and clinical manifestations of dysregulated Jak–STAT signaling, which should ultimately lead to increased development and implementation of pathway-targeting drugs. Thus, building on the glittering legacy of the first 25 years, modern Jak–STAT research is poised to carry on delivering transformative insights about human health and disease and, more broadly, about the nature of cellular communication and gene expression.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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The authors declare competing financial interests: details are available in the online version of the paper.

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