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Review

Mechanisms encoding STAT functional diversity for context-specific inflammatory responses

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Cells integrate complex cytokine cues and other inflammatory stimuli through activation of the signal transducers and activators of transcription (STAT) family of transcription factors to drive the appropriate anti-microbial, inflammatory, and resolving functions. Here, we discuss recent progress in our understanding of mechanisms supporting STAT functional diversity. Signaling component availability and the strength of receptor and STAT interactions emerge as important determinants of immune function. The resultant dynamics of STAT activation, together with stimulus-specific variation in STAT post-translationally modified states, will impact downstream binding partners to support transcription of distinct gene subsets. Understanding how context-dependent STAT function is encoded to dictate cytokine specificity, crosstalk, and control of inflammation will guide therapeutic efforts to selectively perturb STAT-regulated responses.

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Introduction

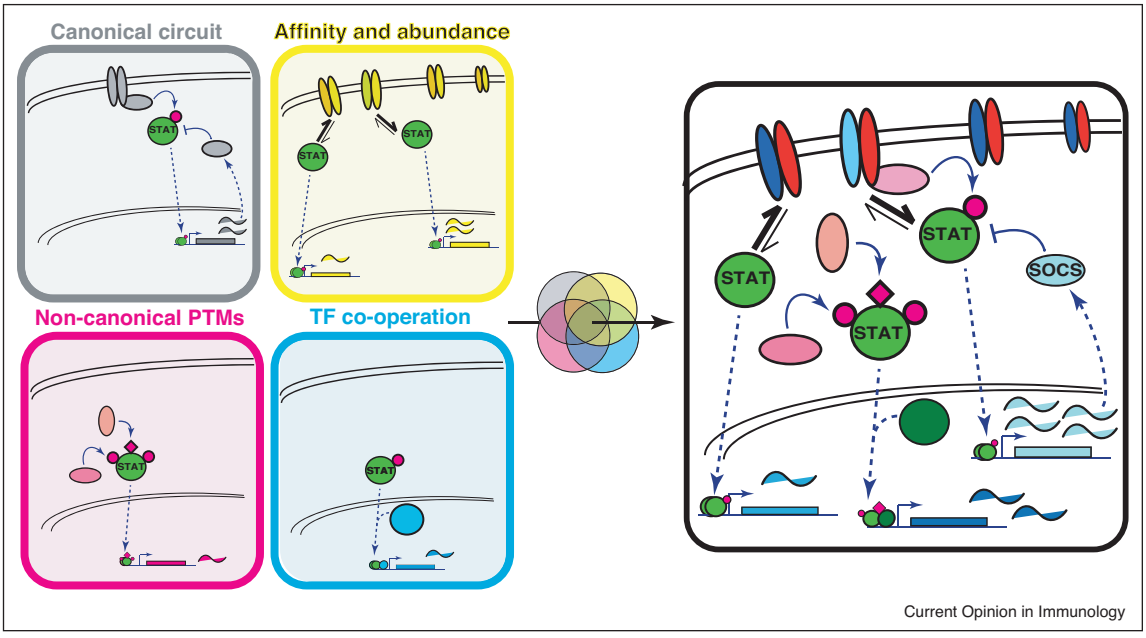
Infection and return to homeostasis are characterized by dynamic shifts in the balance of pro-inflammatory and anti-inflammatory cytokines. Cells integrate these cytokine stimuli through the activation of the signal transducers and activators of transcription (STAT) transcription factors (TFs) to induce the appropriate transcriptional programs. This process determines the quality and quantity of anti-microbial, inflammatory, and resolving cellular functions. STATs convey information about the specific environmental context, including the concentration and combinations of cytokines, other inflammatory stimuli and previous cellular exposure. A small number of STATs drive diverse cytokine-specific transcriptional progams and the same cytokine can lead to varied STAT activation and gene expression outcomes. Thus, a challenge facing the immune system and efforts to manipulate STAT function, is how context-specific STAT function is encoded to direct cytokine specificity, cytokine crosstalk, and control of inflammation.

The general cellular signaling processes allowing cytokines to shape inflammation and resolution through the JAK-STAT pathway have been extensively reviewed [1,2]. Here, we focus on recent progress describing mechanisms of STAT-mediated integration of complex cytokine cues. We focus on three facets of signal integration: STAT activation dynamics, how post-translational modifications (PTMs) tune STAT function, and how cooperating signaling pathways, in particular the activity of additional TFs or kinases, shape STAT-dependent responses (Figure 1). We conclude by discussing approaches aiming at systematically investigating the post-translational events that result in context-specific expression of inflammatory genes.

Cytokine-specific regulation of STAT dynamics

Quantitative studies have revealed temporal characteristics of STAT activation, such as signal duration, that encode stimulus-specificity and the induction of specific target genes. STAT3 and STAT1 translocation and phosphorylation dynamics vary based on the activating cytokine stimulus and can drive a range of pro-inflammatory and anti-inflammatory gene expression profiles. Thus, mechanisms shaping their context-specific regulation and function are of particular interest. For instance, Wilmes and colleagues found that IL-27 induced more sustained STAT1 tyrosine (Y701) phosphorylation than IL-6 while either cytokine stimulated a comparable peak and duration of STAT3 Y705 phosphorylation [3•]. Differences in STAT1 phosphorylation were attributed to receptor-STAT binding properties, with strong STAT1

Figure 1



Multiple mechanisms diversifying STAT function. The 'canonical circuit' involves JAK-mediated tyrosine phosphorylation of a STAT TF, subsequent translocation into the nucleus, and gene expression, all limited by the induction of negative regulators. 'Affinity and abundance' dictates cytokine receptors available for signaling, STAT-receptor and receptor-receptor subunit affinity, and subsequent STAT activation dynamics. 'Non-canonical PTMs' includes non-canonical phosphorylations, as well as other PTM chemistries/modalities discussed. 'TF co-operation' illustrates interactions between the STAT of interest with other TFs or transcriptional machinery. Combined (right) these mechanisms enable diverse transcriptional responses to a range of various cytokine stimulation(s).

binding to IL-27Rα and sustained STAT1 activation supporting a unique gene expression profile (Figure 1, yellow panel). This is consistent with work from Hirahara and colleagues, who compared IL-6 and IL-27 driven transcriptional outputs. They showed that STAT1 is the principle driver of transcriptional specificity, while STAT3 dictated overall output and STAT1 binding to chromatin [4]. Thus, receptor-specific dynamics of STAT phosphorylation and formation of STAT heterodimers, which have been reviewed related to their role in directing cytokine targets [5], are likely a common route to stimulus-specific STAT function.

Two recent studies demonstrated that modulation of ligand binding parameters for a given cytokine-receptor can uncouple STAT1 and STAT3 activation dynamics and downstream immunological function [6,7••]. Martines-Fabregas *et al.* showed that IL-6 affinity for the gp130 receptor impacts STAT1 Y701 phosphorylation to a greater degree than STAT3 Y705 phosphorylation, tipping the balance of STAT1 and STAT3 activation and, ultimately, T helper cell functional polarization efficiency [6]. The authors showed that STAT1 activation was also more sensitive to the number of phosphorylated tyrosine residues on gp130, compared to STAT3. Saxton and colleagues used a structure-based design to create partial agonist analogs for the IL-22 receptor complex (IL-22Rα–IL-10Rβ) that produced STAT3-biased responses, resulting in reduced activation of STAT1, compared to WT IL-22 [7••]. The STAT3-biased variant uncoupled expression of STAT1 and STAT3 target genes to promote tissue recovery in the absence of inflammation. Notably, the extent of STAT3 bias was tissue specific and correlated with expression levels of IL-10Rβ, suggesting that opposing inflammatory and tissue repair functions of a given cytokine can be targeted in a tissue-selective manner.

Changes in receptor subunit expression can also impact cytokine signaling crosstalk. Gonnord and colleagues showed that limiting amounts of the shared gamma chain receptor subunit conferred an asymmetric signal transduction bias for co-receptors with higher affinity for gamma chain [8]. Treatment with IL-7, or increased expression of IL-7Rα, sequestered gamma chain thereby reducing IL-4 or IL-21 responses regardless of the order of administration. The authors proposed that in complex inflammatory environments a hierarchy of cytokine responsiveness through differential subunit affinities establishes asymmetric crosstalk possibilities. Given that

shared cytokine receptor subunit usage is common, asymmetric crosstalk may be shaped by variation in the abundance of receptor components across tissue microenvironments. In addition to microenvironments, genetic variation and previous cellular exposures will impact both receptor levels and the relative availability of JAK/STAT signaling components to influence cellular responsiveness to various cytokines (reviewed in Villarino *et al.* [2]) and cytokine-mediated immunity (reviewed in Kallal *et al.* [9]).

Stimulus-specific STAT dynamics rely on stimulus-specific induction of negative feedback molecules, including expression and post-translational activation of suppressor of cytokine signaling (SOCS) proteins [10], protein inhibitors of activated STAT (PIAS) molecules [11], tyrosine phosphatases [12], and deubiquitinating enzymes (ubiquitin-specific proteases; USPs) [13] (Figure 1, grey panel). While is it is generally accepted that the function of diverse inhibitory molecules varies depending on the stimulus involved, efforts to directly address the role of these feedback mechanisms in shaping cytokine-specific STAT activity or crosstalk between cytokines have largely focused on SOCS proteins [1]. For example, IL-6-specific induction of SOCS3 results in transient STAT3 Y705 phosphorylation, compared to a more sustained IL-10 induced STAT3 response, where SOCS3 is critical for the divergent STAT3 and STAT1 activation profiles and functions of IL-6, IL-10, and IFNβ [14–16]. SOCS-dependent feedback alone is not sufficient for appropriate inflammatory function. Gruber *et al.* described a human STAT2 missense mutation that resulted in lethal autoinflammation. The defect in proper inflammation control was not due to enhanced early STAT2 signaling and transcriptional activity, but instead due to failed STAT2-mediated recruitment of the deubiquitinating enzyme USP18 to the type I IFN receptor, leading to sustained signaling [17]. Disease-associated polymorphisms in putative regulatory regions for STATs and STAT-regulating proteins, as well as cell state-dependent expression of receptors and STATs, are also likely to influence the degree of negative feedback, where even subtle signaling changes may have a robust impact on cellular decision-making. Mokashi *et al.* used a microfluidic approach to provide evidence that negative feedback mechanisms support the integration of the time-varying nature of cytokine cues for appropriate cellular decisions, with production rates of inhibitory proteins shaping signaling behaviors in response to either persistent or increasing TNF exposure [18]. Thus, our understanding of STAT-mediated cytokine signal integration and dysregulation of inflammation would greatly benefit from more efforts aiming at systematically investigating how temporal STAT activation patterns predict stimulus-specific inflammatory function and the negative regulatory mechanisms that shape these dynamic features.

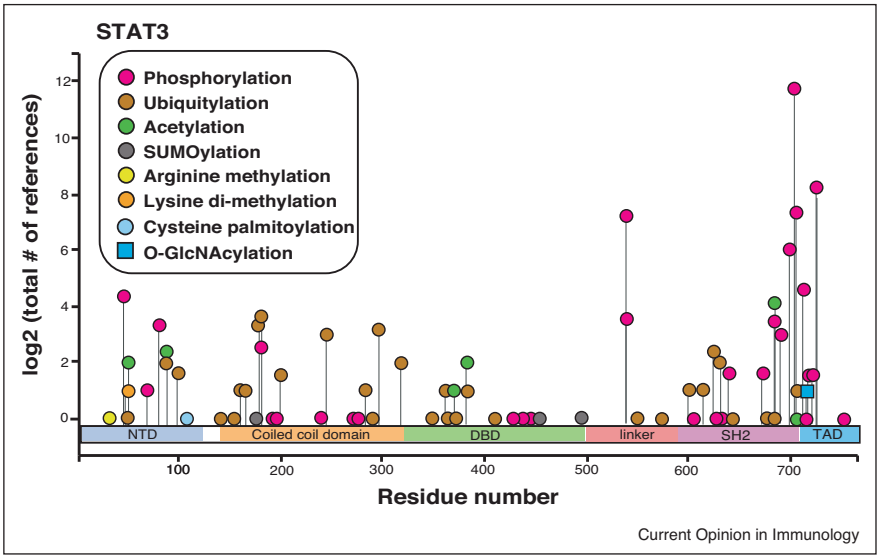
Post-translational modifications of STAT transcription factors

Quantification of STAT activation has traditionally relied on measuring 'canonical' tyrosine phosphorylation (i.e. STAT3 Y705) [2]. However, STATs are extensively decorated with PTMs (Figure 1, pink panel) and major gaps remain in our understanding of how other PTMs influence STAT function. PhosphositePlus.org, a data repository for PTMs, shows STAT1/2/3/4/5a and 5b possess 81 modified sites combined (Figure 2) [19]. This suggests that the STATs exist in an array of post-translationally modified states, where each 'proteoform' may have specific binding partners and may activate distinct subsets of genes. There also exists a 'local crosstalk' potential where different PTMs on the same STAT molecule may influence one another, adding another level of regulation (Reviewed in Leutert *et al.* [20]). Considering that the 'histone code' is comprised of numerous, functionally distinct combinations of PTMs on histone proteins (Reviewed in Huang *et al.* [21]), it seems reasonable to hypothesize the diversity of STAT-mediated gene expression responses may also be conferred through their own PTM code.

S727 of STAT1a is an example of PTM-mediated control of transcription through changes in complex formation, and a potential instance of PTM crosstalk. After phosphorylation of Y701 and translocation into the nucleus, STAT1a is phosphorylated at a separate site, S727, when bound to chromatin [22,23]. STAT1a S727 phosphorylation controls gene loci residence time in response to IL-6 [24••], and promotes the recruitment of histone acetylation machinery for transcriptional activation of IFNγ-responsive genes [25,26]. Gupte and coworkers found that STAT1a S727 phosphorylation, and the resultant IFNγ-responsive transcriptional program, was attenuated in macrophages lacking the ADP-ribosyltransferase PARP1 [27•]. The authors showed that mutation of an ADP-ribosylation site in the DNA binding domain resulted in less stringent DNA motif binding by STAT1a, whereas mutation of the transactivation domain ADP-ribosylation site reduced phosphorylation of S727 but not of Y701. Loss of S727 phosphorylation correlated with decreases in H3K27 acetylation and a reduction in IFNγ-stimulated gene expression. This study demonstrates how non-canonical STAT PTMs can influence one another, and modulate downstream transcriptional activities.

Canonical STAT tyrosine phosphorylation can also be affected by understudied PTM chemistries/modalities. Zhang and colleagues describe how the acylation of STAT3 regulates its activation [28••]. The palmitoyltransferase DHHC7 couples a long fatty acid chain to STAT3, promoting its localization to the plasma membrane and propensity for JAK2-mediated Y705 phosphorylation. The acyl protein thioesterase APT2 removes the

Figure 2



Extensive PTM landscape of STAT3. Diagram of STAT3 illustrating the modified residue (x-axis) and the number of literature references detecting the PTM through mass spectrometric analyses or if studied individually (y-axis). Different PTM chemistries/modalities are color coded. NTD, N-terminal domain; DBD, DNA-binding domain; TAD, transactivation domain. Figure adapted from PhosphositePlus.org Oct 2021.

palmitoyl moiety allowing for phospho-STAT3 to enter the nucleus driving its transcriptional program. This palmitoylation cycle was critical for normal regulation of T cell inflammatory function, and peripheral blood cells from patients with inflammatory bowel disease showed higher *DHHC7* and *APT2* mRNA levels. Thus, alternate PTM enzymes represent new potential points of intervention for the treatment of autoinflammatory diseases, though how they are controlled is poorly understood. Untangling these biochemical regulatory networks will require more holistic views of the dynamic and coordinated PTM landscape.

STAT-cooperating pathways in control of inflammation

Protein complexes orchestrate the majority of cellular functions, and their formation is driven largely by subunit availability and PTM states. Cell type-specific and microenvironment-dependent protein expression coupled with the dynamics of STAT activation and PTM state variation will impact STAT binding partners to support transcription of distinct gene subsets (Figure 1, blue panel). Wienerroither *et al.* describe cooperative transcriptional activation by NF-kB and the trimeric ISGF3 complex, consisting of the tyrosine-phosphorylated STAT1/STAT2 heterodimer with IRF9 [29]. The authors demonstrate NF-kB-dependent establishment of active H3K4me3 chromatin marks to selectively prime promoters for STAT engagement to support type I IFN induced antimicrobial gene expression. ISGF3 had a role in recruiting core transcriptional machinery. Goldstein *et al.* showed that, compared to IL-6 stimulation alone, adding IL-1β activated NF-kB to prime enhancer activity and support STAT3 binding [30]. This NF-kB 'assisted loading' of STAT3 was highly enhancer-specific [30], and may be independent of STAT3 Y705 phosphorylation (reviewed in Yang *et al.* [31]). Given that NF-kB is also activated by diverse stimuli involved in inflammation and resolution, it seems likely that the cooperation between NF-kB and STATs should be highly sensitive to the timing of the stimuli activating these pathways.

Differential induction of cooperating TF expression shapes cytokine-specific gene expression and function. Forero *et al.* showed type I IFN-specific induction of *IRF1* altered the responsiveness of a subset of STAT1-regulated genes [32]. Low IFNλ receptor abundance meant that type III IFN activation of STAT1 was not sufficient induce *IRF1* and its gene program. The authors propose that type III IFNs limit both viral spread and inflammation-associated tissue damage, while a transient type I IFN response supports robust recruitment of immune effectors to the infection site. The various pathways that regulate IRF1 to support its roles in pattern recognition and cytokine signaling crosstalk have been recently reviewed [33]. Signaling component availability clearly emerges as an important determinant of cytokine-mediated immune function. Especially considering the importance of IFNλ receptor and IRF1 abundance, as well as evidence that STAT1-STAT2 heterodimers,

STAT2-IRF9 complexes, and trimeric ISGF3 complexes have distinct transcriptional functions [34].

Cytokine-specific regulation of kinases will have both transcriptional and post-transcriptional consequences regarding regulation of STAT target genes. Martines-Fabregas and colleagues identified CDK8 as a negative regulator of STAT3 transcriptional activity, with CDK8 inhibition resulting in increased STAT3 DNA binding and transcriptional activity [24••]. Transcriptomic and phosphoproteomic analyses revealed CDK8 regulates the IL-6-STAT3 transcriptional response by controlling STAT3 gene loci resident time, and through phosphorylation of transcriptional machinery. Of note, proteins with mRNA splicing and export functions were also sensitive to CDK8 inhibition, highlighting the importance of post-transcriptional mechanisms in shaping context-specific STAT-dependent gene expression. Systematic investigation of stimulus-specific kinase activity will be required for a complete understanding of the multifaceted processes guiding distinct immune functions that are associated with STAT-dependent transcription.

Conclusions

Considering the versatile transcriptional programs controlled by the STATs it should be no surprise that complex, context-specific regulation is needed to achieve specificity. Intricate biochemical circuits vary in space and time to eventually converge on these TFs, establishing a diverse repertoire of STAT PTM states and transcriptional complexes. Combined with the overall state of the cell (i.e. protein levels of other master regulator TFs, chromain accessibility patterns, etc.) these elaborate 'signaling-to-transcription networks' likely enable a diverse set of responses from a limited number of proteins. The findings reviewed here are likely the tip of the iceberg for STAT regulatory mechanisms. Achieving a better understanding of this complex regulation and, in doing so, supporting efforts to manipulate host defense and inflammation, will require holistic and interdisciplinary approaches. Technologies like mass spectrometric methods to delineate global signaling dynamics as well as local PTM interplay on the STATs themselves, high-resolution genomic techniques such as nascent RNA-seq, ATAC-seq, or CUT-N-RUN, and computational approaches to support prediction of mechanistic links between biochemical events and select gene profiles will guide us from the 'streetlamp effect', focusing only on canonical phosphorylation events. Unraveling the complexity of context-specific STAT regulation, though challenging, will provide insight into cellular decision making, while identifying new therapeutic opportunities to control pro-inflammatory and anti-inflammatory properties of the immune system.

Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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