JAK-STAT 2:4, e25097; October/November/December 2013; © 2013 Landes Bioscience

Genomic and computational approaches to dissect the mechanisms of STAT3's universal and cell type-specific functions

Andrew Paul Hutchins, Diego Diez, and Diego Miranda-Saavedra*

Bioinformatics and Genomics Laboratory; World Premier International (WPI) Immunology Frontier Research Center (IFReC); Osaka University; Suita, Osaka Japan

Keywords: STAT3, JAK-STAT, ChIP-seq, genomics, bioinformatics, pleiotropy, transcriptional regulatory modules, cancer, inflammation

STAT3 is the quintessential pleiotropic transcription factor with many biological roles throughout development as well as in multiple adult tissues. Its functional heterogeneity is encoded in the range of genome-wide binding patterns that specify different regulatory networks in distinct cell types. However, STAT3 does not display remarkable DNA binding preferences that may help correlate specific motifs with individual biological functions or cell types. Therefore, achieving a detailed understanding of the regulatory mechanisms that endow STAT3 (or any other pleiotropic transcription factor) with such a rainbow of functions is not only a central problem in biology but also a fiendishly difficult one. Here we describe key genomic and computational approaches that have shed light into this question, and present the two current models of STAT3 binding (universal and cell type-specific). We also discuss the role that the local epigenetic environment plays in the selection of STAT3 binding sites.

The Problem of (Understanding) Transcription Factor Pleiotropy

Transcription factors (TFs) bind to short DNA sequences where they combine with other co-factors to regulate the expression of target genes in specific epigenetic and nuclear contexts. Some of the most dramatic effects a TF can have are those related to cellular differentiation. For instance, MyoD alone is capable of trans-differentiating fibroblasts to myoblasts,[1] and a combination of only four TFs (OCT4, SOX2, KLF4, and c-MYC) is sufficient to reprogram terminally differentiated fibroblasts into iPS cells that display an embryonic stem cell-like phenotype.[2] Fibroblasts can also be transformed into tripotent neural precursor cells with a defined cocktail of TFs (BRN2, SOX2, and FOXG1).[3] Clearly a TF's ability to recognize some sort of "code" contained in the DNA region it binds to is essential for the successful execution of gene expression programs. However, the analysis of the DNA sequence preferences of TFs using a variety of high-throughput methods have underlined the general binding degeneracy of TF families,[4-6] indicating that there is no simple relationship between the DNA sequence a TF binds to and the biological program it executes. Therefore, mechanisms other than the mere binding of a TF to DNA must be involved in determining the cell type-specific functions of TFs. Complex models describing the interplay between groups of TFs,[7] as well as the influence of the epigenetic environment on transcription,[8] have been proposed to explain the functional specificity of TFs. However, none of these models are entirely satisfactory or comprehensive, and no broadly applicable rules have been described to explain the mechanisms whereby TFs discriminate and select specific binding sites genome-wide to perform specific functions.

The diversity of cells and tissues where a TF is expressed can serve as an approximation to infer its functional diversity. We may thus classify TFs into one of three categories: (1) TFs whose expression is restricted to a single cell-type (e.g., Oct4 [Pou5f1] is primarily expressed in embryonic stem cells); (2) TFs restricted to a single germ lineage, such as the SoxB1 subfamily of TFs (ectodermal) and STAT4 (primarily mesodermal); and (3) TFs that are ubiquitously expressed, such as most members of the STAT family of TFs (**Fig. 1**). It is this latter category of widely expressed TFs that presents the greatest intellectual challenge as they normally display a large variety of functions (pleiotropy), including opposing functions in distinct cell types, and sometimes within the same cell type too.

In this review we explore the general problem of understanding the regulatory mechanisms of multi-functional TFs by taking STAT3 as a prime example of a pleiotropic TF. Recent work that integrates high-throughput genomics and detailed computational analyses has shed new light into the mechanisms employed by STAT3 to perform completely different biological functions in distinct cell types. These models are specific to STAT3, but the concepts and tools involved in the analyses are applicable to other TFs, thus opening the door to a more thorough mechanistic understanding of TFs with complex functions.

*Correspondence to: Diego Miranda-Saavedra;
Email: diego@ifrec.osaka-u.ac.jp
Submitted: 04/25/2013; Revised: 05/20/2013; Accepted: 05/20/2013
Citation: Hutchins AP, Diez D, Miranda-Saavedra D. Genomic and computational approaches to dissect the mechanisms of STAT3's universal and cell type-specific functions. JAK-STAT 2013; 2:e25097; http://dx.doi.org/10.4161/jkst.25097

The Dazzling Functional Diversity of STAT3

STAT3 is constitutively expressed and its genetic deletion is embryonic lethal,[9] possibly due to an essential role in maintaining pluripotency.[10] This early severe phenotype therefore masks many other functional defects associated with the loss of the STAT3 gene and which have been painstakingly teased apart using cell type-specific knockouts and carefully designed cell culture experiments. For instance, the roles of STAT3 in metabolism are many and varied, including links to obesity and glucose tolerance, as shown by the deletion of STAT3 in the neural system, hypothalamus, pancreatic islets, and adipose cells where it leads to a general impairment of metabolism[11-14] due to the critical role that STAT3 plays downstream of leptin signaling.[15] $Stat3^{S727A/-}$ mice, which have reduced levels of STAT3 transcriptional activity, present growth retardation and a reduction in thymocyte numbers as well as defects in insulin growth factor-1 (IGF-1) and growth hormone (GH).[16] This suggests that STAT3 might be indirectly responsible for promoting their expression, and hints at a positive feedback loop controlling growth as both IGF-1 and GH can activate STAT3.[17,18]

STAT3 is also essential in many developmental pathways. For example, in keratinocytes STAT3 is required for hair production, cell migration, and wound repair,[19] whereas the genetic ablation of STAT3 in cardiomyocytes results in cardiac dysfunction as well as an increased sensitivity to inflammatory stimuli.[20] Moreover, a liver-specific knockout resulted in the suppression of liver regeneration after a partial hepatectomy.[21] The hematopoietic system is perhaps where STAT3 functions have been most intensely studied, although its role in hematopoietic progenitors remains controversial. The genetic deletion of STAT3 in blood progenitors leads to a higher rate of myeloid cell production, particularly macrophages[22] and neutrophils.[23] In this context, STAT3 is likely controlling proliferation and homeostatic control. STAT3 is also required later in hematopoiesis in Flt3L-dependent dendritic cell development,[24] as well as for controlling the apoptosis of conventional dendritic cells.[25] In B lymphocytes, STAT3 is required for the differentiation of IgG B cells;[26] whereas in cytokine-stimulated (IL-6, IL-21, and IL-23) $CD4^+$ T cells STAT3 specifies the differentiation of Th17 cells by binding at multiple sites in the vicinity of key genes, including Il17a and Il17f.[27,28] STAT3 is also important for the immune-suppressive Treg cells through a direct action on Foxp3 expression.[29]

Besides its involvement in development, STAT3 plays central roles in cellular responses to environmental stimuli. On the whole-organ level, an interferon-inducible conditional STAT3 knockout in liver cells results in a loss of correct inflammatory function in the liver.[30] The stimulation of myeloid cells with IL-10 activates STAT3 and has an anti-inflammatory effect,[31-35] whereas STAT3 synergizes with glucocorticoid receptor signaling to regulate inflammation in the pituitary.[36] Likewise, in fibroblasts IL-6 synergizes with IL-17A/NFκB signaling to engage an inflammatory response.[37,38] Opposing effects of STAT3 have also been described within the same cell type: in DCs, the IL-6-mediated pro-inflammatory activity of STAT3 appears to be rapidly induced, followed by a decline, whereas the IL-10-mediated anti-inflammatory response is encoded in the sustained activity of STAT3.[39] This change in duration of STAT3 activity is controlled by SOCS3, which binds to the phospho-Y759 residue of the IL-6 receptor (specifically the gp130 subunit) to inhibit downstream signaling through STAT3, and so blocks IL-6 activity.[40] However SOCS3 does not bind to any analogous residue in the IL-10 receptor,[41,42] and so IL-10 can continue to sustain the activation of STAT3. In addition to the opposing effects of IL-6 and IL-10, complicating this picture further is the action of IL-21 on conventional DCs (a subtype of DCs) where it activates STAT3 to engage apoptosis.[25] These observations in DCs highlight the complexity of STAT3 function: three different cytokines signal through STAT3 to produce three entirely distinct cellular responses in the same cell type.

Given its vast functional diversity it is not surprising that mutations in STAT3 are responsible for a plethora of diseases. These include the Hyper-IgE syndrome, a condition characterized by recurrent colds, pneumonia, eczema, scoliosis, and extreme elevation of IgE.[43] The underlying cause of the Hyper-IgE syndrome remains unclear, and the phenotype is complex with various abnormalities throughout the immune system, particularly in T cell development as these patients present impaired T follicular helper cells[44] and lack both $CD4^+$ and $CD8^+$ T memory cells.[45] Interestingly many of the mutations identified in Hyper-IgE syndrome patients are located in the DNA binding domain of STAT3,[43] and they impair but do not abolish STAT3's DNA-binding ability.[46] In contrast, gain-of-function mutations in STAT3 are rare[47] but they do occur and are linked with cancer. For instance, several different mutations in STAT3 (primarily within the SH2 domain) are associated with inflammatory hepatocellular adenoma and activate transcription in the absence of a cytokine.[48] STAT3 is associated with a wide array of cancers,[49] especially for its role in promoting inflammation within and around the tumor as many tumors show excessive STAT3-mediated inflammation in combination with NFκB signaling.[47,50] Although the exact relationship between STAT3 and the various types of cancer continues to be actively researched ("Is STAT3 a cause or a consequence?"), at the very least in the case of pancreatic ductal adenocarcinoma STAT3 is essential for both tumor initiation and progression[51] where it cooperates with SOX2 to transform foregut basal progenitor cells.[52] Moreover, STAT3 has been widely implicated in inflammatory diseases, such as Crohn disease: the IL-10 knockout mouse[53] is the prototypical mouse model for Crohn disease and when STAT3 is knocked out during hematopoiesis the resulting mice display Crohn disease-like symptoms.[22,33] Finally, an epithelial cell-specific knockout mouse was developed to study Sjögren syndrome, a systemic autoimmune disease where immune cells target exocrine glands, and which is characterized by the lack of Nfkbiz,[54] a universal STAT3 target gene.[55] The involvement of STAT3 in so many pathologies makes it a promising target molecule for anti-cancer treatment[49] despite the general difficulty of inhibiting TFs with small molecules. Decoy oligonucleotides that specifically inhibit STAT3 may hold promise for the treatment of cancer.[56]

In addition to STAT3's role as a cytokine-activated TF, STAT3 has also been found inside mitochondria in multiple cell

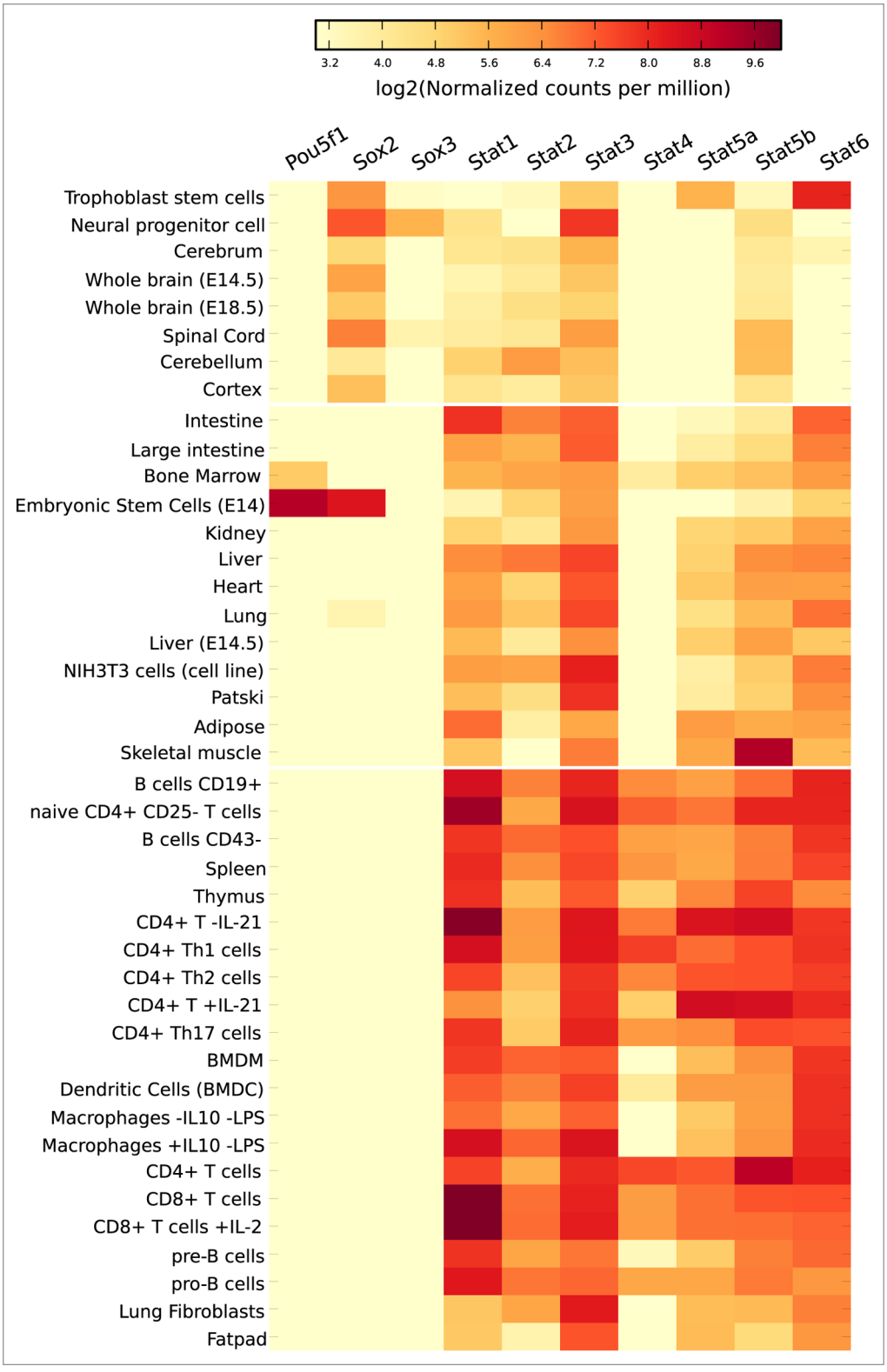

Figure 1. The functional diversity of a TF can be inferred from its expression pattern throughout the body. For instance, the expression of Oct4 (Pou5f1) is primarily restricted to ESCs, whereas most STAT family members are widely expressed in multiple mouse tissues except STAT4, which is primarily mesodermal. Sox2 and Sox3, like STAT4, are also restricted to a single developmental lineage, the ectoderm. RNA-seq data was extracted from the GEO database accessions: GSE20851,[94] GSE20898,[95] GSE29209,[96] GSE29278,[97] GSE31530,[31] GSE33024,[98] GSE34550,[99] GSE36026, GSE39524, GSE39656,[100] GSE39756,[101] GSE40350,[102] GSE40463,[103] GSE42207,[104] GSE42443,[105] and GSE42880.[106]

types.[57] Here STAT3 does not bind mitochondrial DNA as might be expected, but instead forms a complex with GRIM-19 to bind to the electron transport chain, modulate reactive oxygen species production and confer a protective effect against ischemia.[57,58] Additionally STAT3 binds to Stathmin to directly regulate microtubule dynamics in migrating T cells.[59] These fascinating roles of STAT3 are independent of its ability to bind DNA and highlight the functional diversity of STAT3 to act not just as a TF but also as an adaptor protein.

STAT3 as a Model TF to Dissect the Mechanisms Regulating Cell Type-Specific Functions

Besides its large number of documented functions, one important aspect of STAT3 that makes it an ideal system for dissecting the working mechanisms of pleiotropic TFs is its two-step mode of activation upon cytokine stimulation, which results in directly measurable effects. Therefore, the activity of STAT3 can be controlled as a natural switch, and with careful manipulation of the culture environment its activity can be regulated. This is a very important advantage over many other TFs where elaborate experiments using artificial switches need to be performed to modulate their activity.

The DNA-Binding Specificity of STAT3 Cannot Be Correlated with Specific Functions or Cell Types

STAT3 is known to exist in two isoforms, α and β. The former represents the standard version of the molecule, whereas the β isoform is characterized by a C-terminal truncation of the transactivation domain. The β isoform was originally suspected to have dominant-negative effects, but it is actually capable of rescuing STAT3 α embryonic function, and mice survive until birth.[60] However, STAT3 α knockout mice die rapidly after birth, indicating that the post-natal functions of STAT3 require the α isoform. STAT3 α knockout mice also show a complex difference in effecting an inflammatory response in tissue-specific knockouts.[60] Crucially the DNA-binding domain of STAT3 β is identical to that of the α isoform, and can activate the same sets of target genes in most situations.[60]

It has long been suspected that STAT3 regulates different target genes in distinct cell types as previously illustrated for a limited number of genes (summarized by Levy and Lee[61]). The application of ChIP sequencing (ChIP-seq) has underlined the highly divergent cell type-specific binding patterns of STAT3 in various cell types, which in turn reflect the distinct functions of STAT3 in the body.[55] Cell type-specific binding patterns have also been described for other TFs and to different degrees. For instance, out of ~20 000 SOX2 binding sites in embryonic stem cells (ESCs) and neural progenitor cells, only 1200 such sites overlap,[62] while in a more extreme example, only three SMAD3 binding sites overlap between pro-B cells, ESCs and myotubes.[63] Most members of the STAT family bind to a sequence known as the "GAS motif" ("TTCCnGGAA"),[64] a notable exception being STAT6 which has a preference for "TTCCnnGGAA".[65] The analysis of thousands of in vivo STAT3-binding sites from ChIP-seq studies performed in ESCs, AtT-20 cells (pituitary-like), $CD4^+$ T cells, and macrophages showed that variations of the GAS motif alone are incapable of identifying specific functions in these cell types.[55] Moreover, these divergent binding preferences are indistinguishable from those of other STAT family members,[64,65] a characteristic shared with many other TF families.[6] Therefore, alternative STAT3 binding sequences (particularly TGCnnnGAA and TTAnnnGAA) probably represent surrogate methods for STAT3 recruitment.[55] Additional complexity comes from the ability of STAT3 to heterodimerize with other STAT family members, thereby displaying preferences for distinct consensus binding sites, or presenting novel surfaces for co-factors to bind to. Indeed, it has long been known that STAT3 and STAT5 can form heterodimers with a binding preference for distinct DNA sequences,[66] and the heterodimerization of STATs has been suggested to explain the vast functional diversity of STAT family members.[67] Unfortunately no systematic analysis has yet been performed on heterodimeric STAT complexes, while other examples exist where two STATs engage in competitive binding. For example, STAT3 and STAT5 have opposing roles in Th17 cells and mutually compete for the same binding sites.[28] So, if variations of the canonical GAS motif cannot broadly account for the functional specificity of STAT3 in distinct cell types, what are the mechanisms responsible for STAT3's widely divergent binding patterns and functions in these cells?

STAT3 Has Two Modes of Binding: Universal and Cell Type-Specific

Besides the widely divergent genomic binding patterns of STAT3 in ESCs, AtT-20 cells, $CD4^+$ T cells, and macrophages, our analysis also unveiled a "shared overlap" of 35 non-random in vivo binding sites in these four cell types.[55] What makes this set of 35 (evolutionarily conserved) binding sites so relevant is that the genes that STAT3 appears to be regulating are essential for STAT3 signaling. Therefore we describe two distinct modes of STAT3 binding: one that is universal to all cells ("universal" or "cell type-independent"), and various cell type-specific binding modes whereby STAT3 mediates diverse functions in distinct cells by operating cell type-specific regulatory networks.

Within the universal core of 35 STAT3 binding sites we found that STAT3 binds to its own transcription start site (TSS) in all four cell types to promote its own transcription (**Fig. 2A**), and that it also regulates genes important for functions downstream of itself. For instance, STAT3 is recruited to SOCS3 (**Fig. 2B**), which blocks the IL-6 pro-inflammatory response in macrophages by binding to the IL-6 receptor.[42] SOCS3 similarly moderates the pro-inflammatory response in DCs[39] and is also upregulated by STAT3 in mammary cells.[68] In ESCs, SOCS3 regulates LIF/STAT3 signaling[69] and is thought to be a STAT3 target in many other settings.[70] Additionally, STAT3 binds to Bcl3 in all four cell types: in interleukin 10-stimulated macrophages Bcl3 suppresses TNF-α expression,[71] whereas in a myeloma cell line Bcl3 has a pro-apoptotic effect downstream of IL-6.[72] The protein tyrosine phosphatase Ptpn1, a well-known

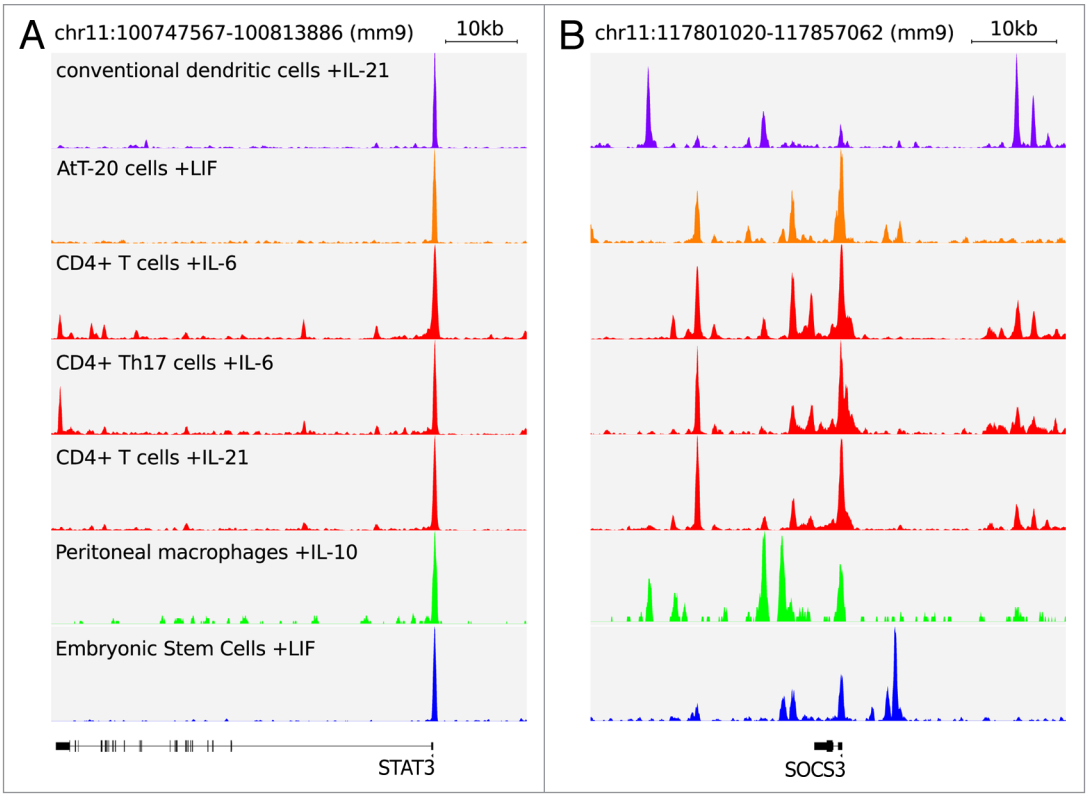

Figure 2. STAT3 binds to a universal core of 35 binding sites to regulate a specific gene set that engages a self-regulatory loop for STAT3 signaling. The examples shown here include: (**A**) STAT3 binding to its own promoter in all cell types examined; and (**B**) STAT3 being recruited to the SOCS3 promoter. STAT3-binding data sets were obtained from the following ChIP-seq libraries: GSE27161,[25] GSE37235,[36] GSE21669,[27] GSE19198,[85] GSE11431,[74] and GSE31531.[31]

negative regulator of JAK-STAT signaling, is likewise regulated by STAT3.[73] STAT3 is also recruited to several other TF genes, including Nfkbiz, Junb, Fos, Irf2, and Bcl6. These findings suggest that the universal core of 35 STAT3 binding sites dictates the self-regulation of STAT3 signaling by: (1) perpetuating STAT3's transcription; (2) functioning as a master regulator of other TFs working downstream of STAT3; (3) stimulating the transcription of cytoplasmic enzymes that control STAT3's activity; and (4) ensuring a robust cellular division program and the maintenance of a stable cell type.[55]

The Reconstruction of Transcriptional Regulatory Modules Sheds Light on STAT3's Regulatory Mechanisms

The vast majority of STAT3-binding sites are cell type-specific, and therefore it is reasonable to assume that distinct regulatory networks are responsible for the various functions of STAT3.[55] One attractive model to explain these cell type-specific binding patterns is the local assembly of groups of TFs and co-factors around STAT3 to form distinct transcriptional regulatory modules (TRMs) that provide cell type-specific activity. This model has been shown to work in several biological systems, including ESCs, where the TRM centers on the core TFs Pou5f1 (OCT4), SOX2, and NANOG[74] but also contains KLF4, SMAD1, ESRRB, and STAT3. In macrophages, PU.1 partners with CEBPa, CEBPg, and AP-1,[75] while in B cells PU.1 forms a regulatory network with E2A, EBF1, and FOXO1 to define a core TRM that determines the B cell phenotype.[75,76]

The identification of TRMs is a difficult task since extensive prior information is required to prioritize candidates for experimental validation. A typical cell may express several hundreds of the ~1400 sequence-specific TFs,[77] all of which can potentially form stable interactions with important co-factors such as histone epigenetic regulators (this is particularly true in the enhanceosome and TF-collective models[7]). We have developed a computational method for the systematic identification of TRMs that works by integrating TF genome-wide locations (from ChIP-seq experiments) with analysis of TF motif enrichment, cell type-specific expression data and protein-protein interactions. We applied our method, called rTRM, to identify the TRMs that endow STAT3 with both universal and cell type-specific functions.[55] We found that in the universal STAT3 TRM, STAT3 forms a specific network with MYC and E2F1, and experimentally showed that E2F1 not only co-localizes with STAT3 in macrophages in over a dozen genomic sites but that it is also prebound at these sites in the absence of cytokine stimulation. This suggests a temporal regulatory code that might help STAT3 locate specific binding sites in the genome (**Fig. 3**). The TRMs reconstructed for the cell type-specific binding patterns of STAT3 suggest that

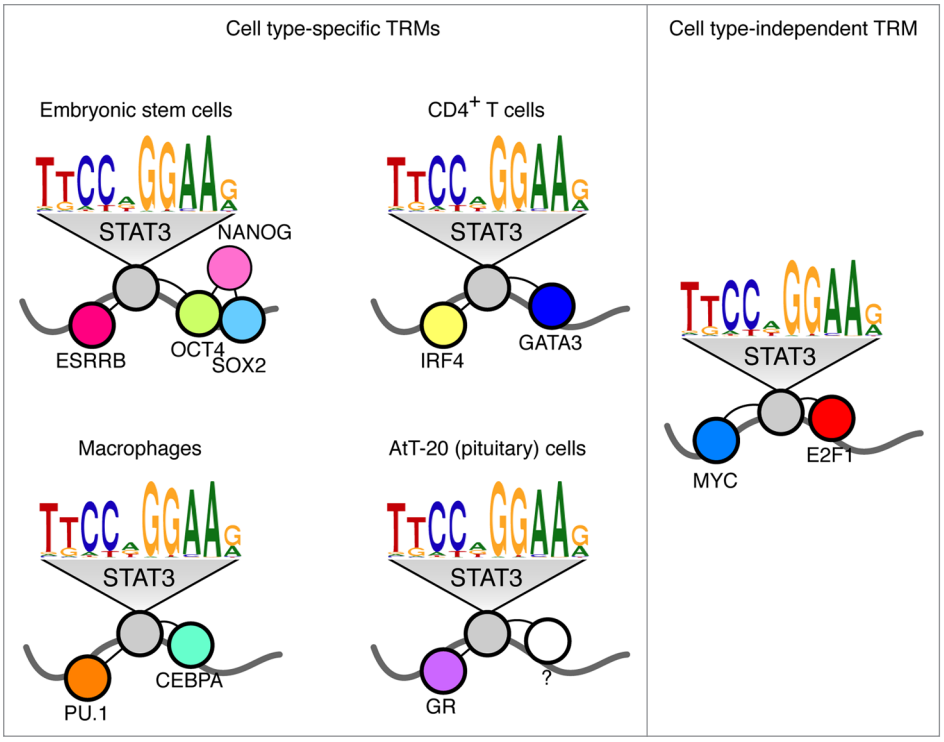

Figure 3. STAT3 combines with specific factors to regulate universal and cell type-specific functions. STAT3 always recognizes the GAS motif in DNA (or slight variations thereof), but co-operates with other TFs and co-factors in what are called transcriptional regulatory modules (TRMs). TRMs include both cell type-specific and general TFs, and are largely responsible for the various functions of STAT3 in different cell types.

STAT3 specifically combines with different factors to perform different functions in distinct cell types (**Fig. 3**).

STAT3 itself is the subject of a large number of post-translational modifications, and specific residues have been implicated in regulating STAT3 homodimer formation as well as in selecting the partner TFs and therefore the TRMs that STAT3 assembles into. For example, in addition to activatory phosphorylations, particularly Y705 and S727, the p300-mediated acetylation of K685 is required to form stable homodimers[78] and for DNMT1 recruitment.[79] Acetylation of K49 and K87 are required for STAT3 to interact with p300.[80,81] K140 is also methylated and appears to negatively regulate a specific subset of genes,[82] presumably by influencing the TRM that assembles around STAT3. Finally, STAT3 and NFκB have a close relationship, particularly in cancer.[47] Here, unphosphorylated STAT3 can bind to p65/RelA and is recruited to NFκB elements in the DNA to activate gene expression in the absence of cytokine signals.[83,84]

The exact role of these post-translational modifications in the context of specific cell types will be an important field of future study. Unfortunately, genome-wide ChIP-seq experiments to detect STAT3 binding have so far used either a pan-STAT3 antibody,[25,31,74,85] a combination of pan-STAT3 and antiphospho-Y705 antibodies,[36] or an antiphospho-S727 STAT3 antibody.[27] As such the genome-wide roles of STAT3 acetylation, methylation and other post-translational modifications remain enigmatic.

Reconstructing TRMs is extremely valuable when access to experimental data sets is limited, or when no regulatory proteins have been identified. One specific advantage of rTRM over other methods is that rTRM integrates protein-protein interaction data sets, which not only provide an additional filtering step but also encapsulate the well-known biological fact that proteins need to physically interact with other proteins to perform specific tasks. Moreover, since protein-protein interfaces evolve faster than protein folds, it is not surprising that the combinatorial potential of proteins into specific modules (TRMs) underlies the functional repertoire displayed by pleiotropic TFs like STAT3.[86]

The Role of the Local Epigenetic Environment in Regulating the Binding and Activity of STAT3

Computational predictions in the mouse genome have thrown a bewildering 1.3 million putative STAT3 binding sites.[70] Still, using a conservative approach by considering only canonical STAT3 binding sites (TTCCnGGAA) yields ~130 000 putative binding sites, a figure much larger than the thousands of genomic sites that STAT3 occupies in vivo as informed by ChIP-seq experiments.[25,27,31,36,74,85] Our view is that STAT3 is recruited to sites that are not only in an "open chromatin" state, but which possibly are also "primed" specifically for STAT3. For instance we have reported that E2F1 is already bound at genomic sites of future STAT3 recruitment, both in untreated and in IL-10 stimulated macrophages.[55] This observation and the rapid activation of many STAT3 target genes (sometimes taking as little as tens of minutes to maximal mRNA production) suggest that STAT3 is not a pioneer factor in the same way as the FoxA family members,[87] but instead is taking advantage of pre-defined cell type-specific modules already present at specific enhancers. However, in other biological systems STAT3 can also modulate the local epigenetic environment directly by recruiting the histone acetyl transferase p300 and thus promote gene expression,[88] or through SIN3A, a transcriptional repressor that recruits histone deacetylases to remove acetyl groups from histones.[89] STAT3 can also recruit the DNA methyltransferase DNMT1 to bring about DNA methylation and so silence gene expression.[79,90] For these reasons, we conclude that it would be fair to categorize STAT3 as an "opportunistic pioneer" TF, i.e., one that initially takes advantage of pre-existing enhancers, but which is also capable of establishing its own transcriptional

network by modifying the local chromatin environment with the help of accessory enzymes.

The ability of STAT3 to bind to pre-existing enhancers is reminiscent of key genomic studies of the macrophage pro-inflammatory response where it has been shown that the macrophage developmental factor PU.1 remains bound in activated macrophages to provide a spectrum of possible transcriptional responses. Lipopolysaccharide (LPS) stimulation of macrophages leads to the recruitment of p300 to sites where PU.1 is already pre-bound to activate pro-inflammatory genes,[91] indicating that PU.1 is thus maintaining the local chromatin in a permissive state to allow the rapid induction of gene expression. However, this model has recently been challenged by the identification of the new class of "latent enhancers", which lack any epigenetic marks typical of enhancers (particularly H3K4me1, H3K4me3, and H3K27ac; specific combinations of histone epigenetic marks are better predictors of enhancers than individual marks[92]) before activation and only recruit lineage-specific TFs in the presence of a triggering stimulus.[93] One possible explanation for the working mechanism of latent enhancers is that early-responding genes are pre-bound by cell type-determining TFs such as PU.1, and that upon LPS activation NFκB binds to these sites to rapidly induce gene transcription. In a second series of events, NFκB may establish its own regulatory network by remodeling the cell's epigenetic landscape and activating a distinct set of genes.[93] It will be fascinating to see if this mechanism also applies to STAT3 and PU.1 is actually marking sites for subsequent STAT3 occupancy during IL-10 stimulation. Indeed, preliminary work in our laboratory suggests that in macrophages PU.1 co-localizes with STAT3 in ~25% (400/1723) of the STAT3 binding sites in macrophages.[31,75,93]

Concluding Remarks and Perspectives

Understanding the multiple regulatory mechanisms of a pleiotropic TF like STAT3, and how these generate the vast spectrum of functions across the body is a problem of byzantine complexity. Although many models have been proposed to explain the cell type-specific binding of TFs, no single, general, model can yet explain how the many functions of pleiotropic TFs are accomplished. Here we have reviewed the case of STAT3, whose genomic binding patterns are predominantly cell type-specific. However, STAT3 also binds to a small, non-random, set of 35 binding sites shared across multiple cell types and which appear to encode a mode of auto-regulation for STAT3 signaling. Therefore we propose two distinct modes for STAT3 binding: one that is universal to all cells, and various cell type-specific binding modes whereby STAT3 mediates diverse functions in distinct cells by operating cell type-specific regulatory networks. The models described here (**Fig. 3**) are specific to STAT3, but the concepts and tools involved in the analyses can be applied to dissect the regulatory mechanisms of other TFs, thus opening the door to a more thorough mechanistic understanding of TFs with complex functions.

As more ChIP-seq data accumulate for specific TFs in multiple cell types, coupled with functional genomic analysis, it is likely that a set of models will emerge to explain the regulatory mechanisms of pleiotropic TFs. STAT3 is a fascinating TF to study as well as an excellent model system, not only because it works as a natural biological switch but also because of its involvement in such a wide array of essential biological processes. Furthermore, since STAT3 is implicated in many diseases, particularly cancer and inflammation, achieving a detailed mechanistic understanding of how STAT3 regulates its transcriptional programs will have important consequences for the design of disease-specific therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr Ralf Jauch for valuable discussions, Mr Shaq Liu for excellent systems support, and Ms Mineko Tanimoto for secretarial support. Work in the D Miranda-Saavedra laboratory is funded by the Japan Society for the Promotion of Science (JSPS) through the WPI-IFReC Research Program and a Kakenhi grant; the Kishimoto Foundation; and the ETHZ-JST Japanese-Swiss Cooperative Program.

References

1. Davis RL, Weintraub H, Lassar AB. Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell 1987; 51:987-1000; PMID:3690668; http://dx.doi.org/10.1016/0092-8674(87)90585-X
2. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126:663-76; PMID:16904174; http://dx.doi.org/10.1016/j.cell.2006.07.024
3. Lujan E, Chanda S, Ahlenius H, Südhof TC, Wernig M. Direct conversion of mouse fibroblasts to self-renewing, tripotent neural precursor cells. Proc Natl Acad Sci U S A 2012; 109:2527-32; PMID:22308465; http://dx.doi.org/10.1073/pnas.1121003109
4. Badis G, Berger MF, Philippakis AA, Talukder S, Gehrke AR, Jaeger SA, et al. Diversity and complexity in DNA recognition by transcription factors. Science 2009; 324:1720-3; PMID:19443739; http://dx.doi.org/10.1126/science.1162327
5. Berger MF, Badis G, Gehrke AR, Talukder S, Philippakis AA, Peña-Castillo L, et al. Variation in homeodomain DNA binding revealed by high-resolution analysis of sequence preferences. Cell 2008; 133:1266-76; PMID:18585359; http://dx.doi.org/10.1016/j.cell.2008.05.024
6. Jolma A, Yan J, Whitington T, Toivonen J, Nitta KR, Rastas P, et al. DNA-binding specificities of human transcription factors. Cell 2013; 152:327-39; PMID:23332764; http://dx.doi.org/10.1016/j.cell.2012.12.009
7. Spitz F, Furlong EE. Transcription factors: from enhancer binding to developmental control. Nat Rev Genet 2012; 13:613-26; PMID:22868264; http://dx.doi.org/10.1038/nrg3207
8. Xu J, Smale ST. Designing an enhancer landscape. Cell 2012; 151:929-31; PMID:23178114; http://dx.doi.org/10.1016/j.cell.2012.11.007
9. Takeda K, Noguchi K, Shi W, Tanaka T, Matsumoto M, Yoshida N, et al. Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality. Proc Natl Acad Sci U S A 1997; 94:3801-4; PMID:9108058; http://dx.doi.org/10.1073/pnas.94.8.3801
10. Niwa H, Burdon T, Chambers I, Smith A. Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. Genes Dev 1998; 12:2048-60; PMID:9649508; http://dx.doi.org/10.1101/gad.12.13.2048
11. Cernkovich ER, Deng J, Bond MC, Combs TP, Harp JB. Adipose-specific disruption of signal transducer and activator of transcription 3 increases body weight and adiposity. Endocrinology 2008; 149:1581-90; PMID:18096662; http://dx.doi.org/10.1210/en.2007-1148
12. Cui Y, Huang L, Elefteriou F, Yang G, Shelton JM, Giles JE, et al. Essential role of STAT3 in body weight and glucose homeostasis. Mol Cell Biol 2004; 24:258-69; PMID:14673160; http://dx.doi.org/10.1128/MCB.24.1.258-269.2004
13. Gao Q, Wolfgang MJ, Neschen S, Morino K, Horvath TL, Shulman GI, et al. Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and thermal dysregulation. Proc Natl Acad Sci U S A 2004; 101:4661-6; PMID:15070774; http://dx.doi.org/10.1073/pnas.0303992101

14. Gorogawa S, Fujitani Y, Kaneto H, Hazama Y, Watada H, Miyamoto Y, et al. Insulin secretory defects and impaired islet architecture in pancreatic beta-cell-specific STAT3 knockout mice. Biochem Biophys Res Commun 2004; 319:1159-70; PMID:15194489; http://dx.doi.org/10.1016/j.bbrc.2004.05.095
15. Buettner C, Pocai A, Muse ED, Etgen AM, Myers MG Jr., Rossetti L. Critical role of STAT3 in leptin's metabolic actions. Cell Metab 2006; 4:49-60; PMID:16814732; http://dx.doi.org/10.1016/j.cmet.2006.04.014
16. Shen Y, Schlessinger K, Zhu X, Meffre E, Quimby F, Levy DE, et al. Essential role of STAT3 in postnatal survival and growth revealed by mice lacking STAT3 serine 727 phosphorylation. Mol Cell Biol 2004; 24:407-19; PMID:14673173; http://dx.doi.org/10.1128/MCB.24.1.407-419.2004
17. Ram PA, Park SH, Choi HK, Waxman DJ. Growth hormone activation of Stat 1, Stat 3, and Stat 5 in rat liver. Differential kinetics of hormone desensitization and growth hormone stimulation of both tyrosine phosphorylation and serine/threonine phosphorylation. J Biol Chem 1996; 271:5929-40; PMID:8621467; http://dx.doi.org/10.1074/jbc.271.10.5929
18. Zong CS, Chan J, Levy DE, Horvath C, Sadowski HB, Wang LH. Mechanism of STAT3 activation by insulin-like growth factor I receptor. J Biol Chem 2000; 275:15099-105; PMID:10747872; http://dx.doi.org/10.1074/jbc.M000089200
19. Sano S, Itami S, Takeda K, Tarutani M, Yamaguchi Y, Miura H, et al. Keratinocyte-specific ablation of Stat3 exhibits impaired skin remodeling, but does not affect skin morphogenesis. EMBO J 1999; 18:4657-68; PMID:10469645; http://dx.doi.org/10.1093/emboj/18.17.4657
20. Jacoby JJ, Kalinowski A, Liu MG, Zhang SS, Gao Q, Chai GX, et al. Cardiomyocyte-restricted knockout of STAT3 results in higher sensitivity to inflammation, cardiac fibrosis, and heart failure with advanced age. Proc Natl Acad Sci U S A 2003; 100:12929-34; PMID:14566054; http://dx.doi.org/10.1073/pnas.2134694100
21. Haga S, Ogawa W, Inoue H, Terui K, Ogino T, Igarashi R, et al. Compensatory recovery of liver mass by Akt-mediated hepatocellular hypertrophy in liver-specific STAT3-deficient mice. J Hepatol 2005; 43:799-807; PMID:16083985; http://dx.doi.org/10.1016/j.jhep.2005.03.027
22. Welte T, Zhang SS, Wang T, Zhang Z, Hesslein DG, Yin Z, et al. STAT3 deletion during hematopoiesis causes Crohn's disease-like pathogenesis and lethality: a critical role of STAT3 in innate immunity. Proc Natl Acad Sci U S A 2003; 100:1879-84; PMID:12571365; http://dx.doi.org/10.1073/pnas.0237137100
23. Lee CK, Raz R, Gimeno R, Gertner R, Wistinghausen B, Takeshita K, et al. STAT3 is a negative regulator of granulopoiesis but is not required for G-CSF-dependent differentiation. Immunity 2002; 17:63-72; PMID:12150892; http://dx.doi.org/10.1016/S1074-7613(02)00336-9
24. Laouar Y, Welte T, Fu XY, Flavell RA. STAT3 is required for Flt3L-dependent dendritic cell differentiation. Immunity 2003; 19:903-12; PMID:14670306; http://dx.doi.org/10.1016/S1074-7613(03)00332-7
25. Wan CK, Oh J, Li P, West EE, Wong EA, Andraski AB, et al. The cytokines IL-21 and GM-CSF have opposing regulatory roles in the apoptosis of conventional dendritic cells. Immunity 2013; 38:514-27; PMID:23453633; http://dx.doi.org/10.1016/j.immuni.2013.02.011
26. Fornek JL, Tygrett LT, Waldschmidt TJ, Poli V, Rickert RC, Kansas GS. Critical role for Stat3 in T-dependent terminal differentiation of IgG B cells. Blood 2006; 107:1085-91; PMID:16223771; http://dx.doi.org/10.1182/blood-2005-07-2871
27. Durant L, Watford WT, Ramos HL, Laurence A, Vahedi G, Wei L, et al. Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. Immunity 2010; 32:605-15; PMID:20493732; http://dx.doi.org/10.1016/j.immuni.2010.05.003
28. Yang XP, Ghoreschi K, Steward-Tharp SM, Rodriguez-Canales J, Zhu J, Grainger JR, et al. Opposing regulation of the locus encoding IL-17 through direct, reciprocal actions of STAT3 and STAT5. Nat Immunol 2011; 12:247-54; PMID:21278738; http://dx.doi.org/10.1038/ni.1995
29. Laurence A, Amarnath S, Mariotti J, Kim YC, Foley J, Eckhaus M, et al. STAT3 transcription factor promotes instability of nTreg cells and limits generation of iTreg cells during acute murine graft-versus-host disease. Immunity 2012; 37:209-22; PMID:22921119; http://dx.doi.org/10.1016/j.immuni.2012.05.027
30. Alonzi T, Maritano D, Gorgoni B, Rizzuto G, Libert C, Poli V. Essential role of STAT3 in the control of the acute-phase response as revealed by inducible gene inactivation [correction of activation] in the liver. Mol Cell Biol 2001; 21:1621-32; PMID:11238899; http://dx.doi.org/10.1128/MCB.21.5.1621-1632.2001
31. Hutchins AP, Poulain S, Miranda-Saavedra D. Genome-wide analysis of STAT3 binding in vivo predicts effectors of the anti-inflammatory response in macrophages. Blood 2012; 119:e110-9; PMID:22323479; http://dx.doi.org/10.1182/blood-2011-09-381483
32. Murray PJ, Smale ST. Restraint of inflammatory signaling by interdependent strata of negative regulatory pathways. Nat Immunol 2012; 13:916-24; PMID:22990889; http://dx.doi.org/10.1038/ni.2391
33. Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, Förster I, et al. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity 1999; 10:39-49; PMID:10023769; http://dx.doi.org/10.1016/S1074-7613(00)80005-9
34. Lang R, Patel D, Morris JJ, Rutschman RL, Murray PJ. Shaping gene expression in activated and resting primary macrophages by IL-10. J Immunol 2002; 169:2253-63; PMID:12193690
35. Williams L, Bradley L, Smith A, Foxwell B. Signal transducer and activator of transcription 3 is the dominant mediator of the anti-inflammatory effects of IL-10 in human macrophages. J Immunol 2004; 172:567-76; PMID:14688368
36. Langlais D, Couture C, Balsalobre A, Drouin J. The Stat3/GR interaction code: predictive value of direct/indirect DNA recruitment for transcription outcome. Mol Cell 2012; 47:38-49; PMID:22633955
37. Ogura H, Murakami M, Okuyama Y, Tsuruoka M, Kitabayashi C, Kanamoto M, et al. Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. Immunity 2008; 29:628-36; PMID:18848474; http://dx.doi.org/10.1016/j.immuni.2008.07.018
38. Murakami M, Okuyama Y, Ogura H, Asano S, Arima Y, Tsuruoka M, et al. Local microbleeding facilitates IL-6- and IL-17-dependent arthritis in the absence of tissue antigen recognition by activated T cells. J Exp Med 2011; 208:103-14; PMID:21220456; http://dx.doi.org/10.1084/jem.20100900
39. Braun DA, Fribourg M, Sealfon SC. Cytokine response is determined by duration of receptor and signal transducers and activators of transcription 3 (STAT3) activation. J Biol Chem 2013; 288:2986-93; PMID:23166328; http://dx.doi.org/10.1074/jbc.M112.386573
40. Schmitz J, Weissenbach M, Haan S, Heinrich PC, Schaper F. SOCS3 exerts its inhibitory function on interleukin-6 signal transduction through the SHP2 recruitment site of gp130. J Biol Chem 2000; 275:12848-56; PMID:10777583; http://dx.doi.org/10.1074/jbc.275.17.12848
41. Niemand C, Nimmesgern A, Haan S, Fischer P, Schaper F, Rossaint R, et al. Activation of STAT3 by IL-6 and IL-10 in primary human macrophages is differentially modulated by suppressor of cytokine signaling 3. J Immunol 2003; 170:3263-72; PMID:12626585
42. Yasukawa H, Ohishi M, Mori H, Murakami M, Chinen T, Aki D, et al. IL-6 induces an anti-inflammatory response in the absence of SOCS3 in macrophages. Nat Immunol 2003; 4:551-6; PMID:12754507; http://dx.doi.org/10.1038/ni938
43. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT3 mutations in the hyper-IgE syndrome. N Engl J Med 2007; 357:1608-19; PMID:17881745; http://dx.doi.org/10.1056/NEJMoa073687
44. Ma CS, Avery DT, Chan A, Batten M, Bustamante J, Boisson-Dupuis S, et al. Functional STAT3 deficiency compromises the generation of human T follicular helper cells. Blood 2012; 119:3997-4008; PMID:22403255; http://dx.doi.org/10.1182/blood-2011-11-392985
45. Siegel AM, Heimall J, Freeman AF, Hsu AP, Brittain E, Brenchley JM, et al. A critical role for STAT3 transcription factor signaling in the development and maintenance of human T cell memory. Immunity 2011; 35:806-18; PMID:22118528; http://dx.doi.org/10.1016/j.immuni.2011.09.016
46. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature 2007; 448:1058-62; PMID:17676033; http://dx.doi.org/10.1038/nature06096
47. Grivennikov SI, Karin M. Dangerous liaisons: STAT3 and NF-kappaB collaboration and crosstalk in cancer. Cytokine Growth Factor Rev 2010; 21:11-9; PMID:20018552; http://dx.doi.org/10.1016/j.cytogfr.2009.11.005
48. Pilati C, Amessou M, Bihl MP, Balabaud C, Nhieu JT, Paradis V, et al. Somatic mutations activating STAT3 in human inflammatory hepatocellular adenomas. J Exp Med 2011; 208:1359-66; PMID:21690253; http://dx.doi.org/10.1084/jem.20110283
49. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer 2009; 9:798-809; PMID:19851315; http://dx.doi.org/10.1038/nrc2734
50. Lee H, Herrmann A, Deng JH, Kujawski M, Niu G, Li Z, et al. Persistently activated Stat3 maintains constitutive NF-kappaB activity in tumors. Cancer Cell 2009; 15:283-93; PMID:19345327; http://dx.doi.org/10.1016/j.ccr.2009.02.015
51. Li N, Grivennikov SI, Karin M. The unholy trinity: inflammation, cytokines, and STAT3 shape the cancer microenvironment. Cancer Cell 2011; 19:429-31; PMID:21481782; http://dx.doi.org/10.1016/j.ccr.2011.03.018
52. Liu K, Jiang M, Lu Y, Chen H, Sun J, Wu S, et al. Sox2 cooperates with inflammation-mediated Stat3 activation in the malignant transformation of foregut basal progenitor cells. Cell Stem Cell 2013; 12:304-15; PMID:23472872; http://dx.doi.org/10.1016/j.stem.2013.01.007
53. Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993; 75:263-74; PMID:8402911; http://dx.doi.org/10.1016/0092-8674(93)80068-P
54. Okuma A, Hoshino K, Ohba T, Fukushi S, Aiba S, Akira S, et al. Enhanced apoptosis by disruption of the STAT3-IκB-ζ signaling pathway in epithelial cells induces Sjögren's syndrome-like autoimmune disease. Immunity 2013; 38:450-60; PMID:23453632; http://dx.doi.org/10.1016/j.immuni.2012.11.016
55. Hutchins AP, Diez D, Takahashi Y, Ahmad S, Jauch R, Tremblay ML, et al. Distinct transcriptional regulatory modules underlie STAT3's cell type-independent and cell type-specific functions. Nucleic Acids Res 2013; 41:2155-70; PMID:23295670; http://dx.doi.org/10.1093/nar/gks1300

56. Sen M, Thomas SM, Kim S, Yeh JI, Ferris RL, Johnson JT, et al. First-in-human trial of a STAT3 decoy oligonucleotide in head and neck tumors: implications for cancer therapy. Cancer Discov 2012; 2:694-705; PMID:22719020; http://dx.doi.org/10.1158/2159-8290.CD-12-0191
57. Wegrzyn J, Potla R, Chwae YJ, Sepuri NB, Zhang Q, Koeck T, et al. Function of mitochondrial Stat3 in cellular respiration. Science 2009; 323:793-7; PMID:19131594; http://dx.doi.org/10.1126/science.1164551
58. Szczepanek K, Chen Q, Derecka M, Salloum FN, Zhang Q, Szelag M, et al. Mitochondrial-targeted Signal transducer and activator of transcription 3 (STAT3) protects against ischemia-induced changes in the electron transport chain and the generation of reactive oxygen species. J Biol Chem 2011; 286:29610-20; PMID:21715323; http://dx.doi.org/10.1074/jbc.M111.226209
59. Verma NK, Dourlat J, Davies AM, Long A, Liu WQ, Garbay C, et al. STAT3-stathmin interactions control microtubule dynamics in migrating T-cells. J Biol Chem 2009; 284:12349-62; PMID:19251695; http://dx.doi.org/10.1074/jbc.M807761200
60. Maritano D, Sugrue ML, Tininini S, Dewilde S, Strobl B, Fu X, et al. The STAT3 isoforms alpha and beta have unique and specific functions. Nat Immunol 2004; 5:401-9; PMID:15021879; http://dx.doi.org/10.1038/ni1052
61. Levy DE, Lee CK. What does Stat3 do? J Clin Invest 2002; 109:1143-8; PMID:11994402
62. Lodato MA, Ng CW, Wamstad JA, Cheng AW, Thai KK, Fraenkel E, et al. SOX2 co-occupies distal enhancer elements with distinct POU factors in ESCs and NPCs to specify cell state. PLoS Genet 2013; 9:e1003288; PMID:23437007; http://dx.doi.org/10.1371/journal.pgen.1003288
63. Mullen AC, Orlando DA, Newman JJ, Lovén J, Kumar RM, Bilodeau S, et al. Master transcription factors determine cell-type-specific responses to TGF-β signaling. Cell 2011; 147:565-76; PMID:22036565; http://dx.doi.org/10.1016/j.cell.2011.08.050
64. Lin JX, Li P, Liu D, Jin HT, He J, Ata Ur Rasheed M, et al. Critical Role of STAT5 transcription factor tetramerization for cytokine responses and normal immune function. Immunity 2012; 36:586-99; PMID:22520852; http://dx.doi.org/10.1016/j.immuni.2012.02.017
65. Wei L, Vahedi G, Sun HW, Watford WT, Takatori H, Ramos HL, et al. Discrete roles of STAT4 and STAT6 transcription factors in tuning epigenetic modifications and transcription during T helper cell differentiation. Immunity 2010; 32:840-51; PMID:20620946; http://dx.doi.org/10.1016/j.immuni.2010.06.003
66. Novak U, Mui A, Miyajima A, Paradiso L. Formation of STAT5-containing DNA binding complexes in response to colony-stimulating factor-1 and platelet-derived growth factor. J Biol Chem 1996; 271:18350-4; PMID:8702476; http://dx.doi.org/10.1074/jbc.271.31.18350
67. Delgoffe D, Vignali D. STAT heterodimers in immunity: A mixed message or a unique signal? JAK-STAT 2013; 2:e23060; http://dx.doi.org/10.4161/jkst.23060
68. Clarkson RW, Boland MP, Kritikou EA, Lee JM, Freeman TC, Tiffen PG, et al. The genes induced by signal transducer and activators of transcription (STAT)3 and STAT5 in mammary epithelial cells define the roles of these STATs in mammary development. Mol Endocrinol 2006; 20:675-85; PMID:16293640; http://dx.doi.org/10.1210/me.2005-0392
69. Li Y, McClintick J, Zhong L, Edenberg HJ, Yoder MC, Chan RJ. Murine embryonic stem cell differentiation is promoted by SOCS-3 and inhibited by the zinc finger transcription factor Klf4. Blood 2005; 105:635-7; PMID:15358627; http://dx.doi.org/10.1182/blood-2004-07-2681
70. Vallania F, Schiavone D, Dewilde S, Pupo E, Garbay S, Calogero R, et al. Genome-wide discovery of functional transcription factor binding sites by comparative genomics: the case of Stat3. Proc Natl Acad Sci U S A 2009; 106:5117-22; PMID:19282476; http://dx.doi.org/10.1073/pnas.0900473106
71. Kuwata H, Watanabe Y, Miyoshi H, Yamamoto M, Kaisho T, Takeda K, et al. IL-10-inducible Bcl-3 negatively regulates LPS-induced TNF-alpha production in macrophages. Blood 2003; 102:4123-9; PMID:12907458; http://dx.doi.org/10.1182/blood-2003-04-1228
72. Brocke-Heidrich K, Ge B, Cvijic H, Pfeifer G, Löffler D, Henze C, et al. BCL3 is induced by IL-6 via Stat3 binding to intronic enhancer HS4 and represses its own transcription. Oncogene 2006; 25:7297-304; PMID:16732314; http://dx.doi.org/10.1038/sj.onc.1209711
73. Gu F, Dubé N, Kim JW, Cheng A, Ibarra-Sanchez MdeJ, Tremblay ML, et al. Protein tyrosine phosphatase 1B attenuates growth hormone-mediated JAK2-STAT signaling. Mol Cell Biol 2003; 23:3753-62; PMID:12748279; http://dx.doi.org/10.1128/MCB.23.11.3753-3762.2003
74. Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, et al. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell 2008; 133:1106-17; PMID:18555785; http://dx.doi.org/10.1016/j.cell.2008.04.043
75. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol Cell 2010; 38:576-89; PMID:20513432; http://dx.doi.org/10.1016/j.molcel.2010.05.004
76. Lin YC, Jhunjhunwala S, Benner C, Heinz S, Welinder E, Mansson R, et al. A global network of transcription factors, involving E2A, EBF1 and Foxo1, that orchestrates B cell fate. Nat Immunol 2010; 11:635-43; PMID:20543837; http://dx.doi.org/10.1038/ni.1891
77. Wilson D, Charoensawan V, Kummerfeld SK, Teichmann SA. DBD--taxonomically broad transcription factor predictions: new content and functionality. Nucleic Acids Res 2008; 36(Database issue):D88-92; PMID:18073188; http://dx.doi.org/10.1093/nar/gkm964
78. Yuan ZL, Guan YJ, Chatterjee D, Chin YE. Stat3 dimerization regulated by reversible acetylation of a single lysine residue. Science 2005; 307:269-73; PMID:15653507; http://dx.doi.org/10.1126/science.1105166
79. Lee H, Zhang P, Herrmann A, Yang C, Xin H, Wang Z, et al. Acetylated STAT3 is crucial for methylation of tumor-suppressor gene promoters and inhibition by resveratrol results in demethylation. Proc Natl Acad Sci U S A 2012; 109:7765-9; PMID:22547799; http://dx.doi.org/10.1073/pnas.1205132109
80. Ray S, Boldogh I, Brasier AR. STAT3 NH2-terminal acetylation is activated by the hepatic acute-phase response and required for IL-6 induction of angiotensinogen. Gastroenterology 2005; 129:1616-32; PMID:16285960; http://dx.doi.org/10.1053/j.gastro.2005.07.055
81. Ray S, Lee C, Hou T, Boldogh I, Brasier AR. Requirement of histone deacetylase1 (HDAC1) in signal transducer and activator of transcription 3 (STAT3) nucleocytoplasmic distribution. Nucleic Acids Res 2008; 36:4510-20; PMID:18611949; http://dx.doi.org/10.1093/nar/gkn419
82. Yang J, Huang J, Dasgupta M, Sears N, Miyagi M, Wang B, et al. Reversible methylation of promoter-bound STAT3 by histone-modifying enzymes. Proc Natl Acad Sci U S A 2010; 107:21499-504; PMID:21098664; http://dx.doi.org/10.1073/pnas.1016147107
83. Yoshida Y, Kumar A, Koyama Y, Peng H, Arman A, Boch JA, et al. Interleukin 1 activates STAT3/nuclear factor-kappaB cross-talk via a unique TRAF6- and p65-dependent mechanism. J Biol Chem 2004; 279:1768-76; PMID:14593105; http://dx.doi.org/10.1074/jbc.M311498200
84. Yang J, Liao X, Agarwal MK, Barnes L, Auron PE, Stark GR. Unphosphorylated STAT3 accumulates in response to IL-6 and activates transcription by binding to NFkappaB. Genes Dev 2007; 21:1396-408; PMID:17510282; http://dx.doi.org/10.1101/gad.1553707
85. Kwon H, Thierry-Mieg D, Thierry-Mieg J, Kim HP, Oh J, Tunyaplin C, et al. Analysis of interleukin-21-induced Prdm1 gene regulation reveals functional cooperation of STAT3 and IRF4 transcription factors. Immunity 2009; 31:941-52; PMID:20064451; http://dx.doi.org/10.1016/j.immuni.2009.10.008
86. Perica T, Marsh JA, Sousa FL, Natan E, Colwell LJ, Ahnert SE, et al. The emergence of protein complexes: quaternary structure, dynamics and allostery. Colworth Medal Lecture. Biochem Soc Trans 2012; 40:475-91; PMID:22616857; http://dx.doi.org/10.1042/BST20120056
87. Zaret KS, Carroll JS. Pioneer transcription factors: establishing competence for gene expression. Genes Dev 2011; 25:2227-41; PMID:22056668; http://dx.doi.org/10.1101/gad.176826.111
88. Hou T, Ray S, Lee C, Brasier AR. The STAT3 NH2-terminal domain stabilizes enhanceosome assembly by interacting with the p300 bromodomain. J Biol Chem 2008; 283:30725-34; PMID:18782771; http://dx.doi.org/10.1074/jbc.M805941200
89. Icardi L, Mori R, Gesellchen V, Eyckerman S, De Cauwer L, Verhelst J, et al. The Sin3a repressor complex is a master regulator of STAT transcriptional activity. Proc Natl Acad Sci U S A 2012; 109:12058-63; PMID:22783022; http://dx.doi.org/10.1073/pnas.1206458109
90. Thomas NSB. The STAT3-DNMT1 connection. JAK-STAT 2012; 1:257-60; http://dx.doi.org/10.4161/jkst.22436
91. Ghisletti S, Barozzi I, Mietton F, Polletti S, De Santa F, Venturini E, et al. Identification and characterization of enhancers controlling the inflammatory gene expression program in macrophages. Immunity 2010; 32:317-28; PMID:20206554; http://dx.doi.org/10.1016/j.immuni.2010.02.008
92. Fernández M, Miranda-Saavedra D. Genome-wide enhancer prediction from epigenetic signatures using genetic algorithm-optimized support vector machines. Nucleic Acids Res 2012; 40:e77; PMID:22328731; http://dx.doi.org/10.1093/nar/gks149
93. Ostuni R, Piccolo V, Barozzi I, Polletti S, Termanini A, Bonifacio S, et al. Latent enhancers activated by stimulation in differentiated cells. Cell 2013; 152:157-71; PMID:23332752; http://dx.doi.org/10.1016/j.cell.2012.12.018
94. Guttman M, Garber M, Levin JZ, Donaghey J, Robinson J, Adiconis X, et al. Ab initio reconstruction of cell type-specific transcriptomes in mouse reveals the conserved multi-exonic structure of lincRNAs. Nat Biotechnol 2010; 28:503-10; PMID:20436462; http://dx.doi.org/10.1038/nbt.1633
95. Wei G, Abraham BJ, Yagi R, Jothi R, Cui K, Sharma S, et al. Genome-wide analyses of transcription factor GATA3-mediated gene regulation in distinct T cell types. Immunity 2011; 35:299-311; PMID:21867929; http://dx.doi.org/10.1016/j.immuni.2011.08.007
96. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 2011; 29:644-52; PMID:21572440; http://dx.doi.org/10.1038/nbt.1883
97. Shen Y, Yue F, McCleary DF, Ye Z, Edsall L, Kuan S, et al. A map of the cis-regulatory sequences in the mouse genome. Nature 2012; 488:116-20; PMID:22763441; http://dx.doi.org/10.1038/nature11243

98. Bergsland M, Ramsköld D, Zaouter C, Klum S, Sandberg R, Muhr J. Sequentially acting Sox transcription factors in neural lineage development. Genes Dev 2011; 25:2453-64; PMID:22085726; http://dx.doi.org/10.1101/gad.176008.111
99. Hutchins AP, Poulain S, Fujii H, Miranda-Saavedra D. Discovery and characterization of new transcripts from RNA-seq data in mouse CD4(+) T cells. Genomics 2012; 100:303-13; PMID:22884873; http://dx.doi.org/10.1016/j.ygeno.2012.07.014
100. Klattenhoff CA, Scheuermann JC, Surface LE, Bradley RK, Fields PA, Steinhauser ML, et al. Braveheart, a long noncoding RNA required for cardiovascular lineage commitment. Cell 2013; 152:570-83; PMID:23352431; http://dx.doi.org/10.1016/j.cell.2013.01.003
101. Li P, Spolski R, Liao W, Wang L, Murphy TL, Murphy KM, et al. BATF-JUN is critical for IRF4-mediated transcription in T cells. Nature 2012; 490:543-6; PMID:22992523; http://dx.doi.org/10.1038/nature11530
102. Ring AM, Lin JX, Feng D, Mitra S, Rickert M, Bowman GR, et al. Mechanistic and structural insight into the functional dichotomy between IL-2 and IL-15. Nat Immunol 2012; 13:1187-95; PMID:23104097; http://dx.doi.org/10.1038/ni.2449
103. Vahedi G, Takahashi H, Nakayamada S, Sun HW, Sartorelli V, Kanno Y, et al. STATs shape the active enhancer landscape of T cell populations. Cell 2012; 151:981-93; PMID:23178119; http://dx.doi.org/10.1016/j.cell.2012.09.044
104. Chuong EB, Rumi MA, Soares MJ, Baker JC. Endogenous retroviruses function as species-specific enhancer elements in the placenta. Nat Genet 2013; 45:325-9; PMID:23396136; http://dx.doi.org/10.1038/ng.2553
105. Yu Y, Chen Y, Kim B, Wang H, Zhao C, He X, et al. Olig2 targets chromatin remodelers to enhancers to initiate oligodendrocyte differentiation. Cell 2013; 152:248-61; PMID:23332759; http://dx.doi.org/10.1016/j.cell.2012.12.006
106. Mellén M, Ayata P, Dewell S, Kriaucionis S, Heintz N. MeCP2 binds to 5hmC enriched within active genes and accessible chromatin in the nervous system. Cell 2012; 151:1417-30; PMID:23260135; http://dx.doi.org/10.1016/j.cell.2012.11.022