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The role of STATs in proliferation, differentiation, and apoptosis

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Abstract. The spectrum of biological systems which makes use of the signal transducers and activators of transcription (STAT) paradigm extends beyond the interferon system in which it was first discovered to include many other cytokines and agonists. Having catalogued which STATs are activated by each stimulus, investigators have turned their attention to defining the biological processes and the genes regulated by the STAT pathway. These studies are in their early stages. Although many tools have been developed to probe the STAT pathway, e.g., mutant receptors, dominant-negative STATs, chemically dimerizable STATs, and mice lacking STAT proteins, more is known about the biological phenomenon affected than the molecular mechanism or the STAT-regulated genes involved. The cellular events currently believed to utilize STAT-dependent pathways can be grouped according to those which affect cell growth, differentiation, and apoptosis.

Key words. STAT; proliferation; differentiation; opoptosis; oncogenesis.

The role of signal transducers and activators of transcription (STATs) in cell growth

The first STAT was described as a transcription factor involved in the expression of interferon (IFN)-induced genes [1–4]. However, the rapid characterization of other STAT family members, and the discovery that many of these STATs are constitutively activated in transformed cells suggested they have additional roles in regulating cell growth. Under normal circumstances proliferation is tightly controlled by cytokines or growth factors, and STAT transcription factors have been implicated in the signalling mechanisms utilized by such growth regulators. While the consequence of STAT1 or 3 activation can be positive or negative depending on the stimulus and cell type involved, STAT4, 5 and 6 have mainly been described as transducing positive cell growth signals.

STAT1 and proliferation

The ability of IFN-γ to inhibit the growth of a number of cell types is STAT1 dependent. For example, the STAT1-difficient U3A fibroblast line is resistant to the anti-proliferative action of IFN-γ but transfection of STAT1 into these cells restores their sensitivity to IFN-γ growth inhibition [5]. Further studies indicated that STAT1 mediates this inhibition through regulation of the expression of cell cycle inhibitors. The mammalian cell cycle is controlled by cyclins and their catalytic partners, the cyclin-dependent kinases [6] which are subject to negative regulation by two families of cell cycle inhibitors including the WAF1/CIP/KIP and the INK4 proteins [7]. The ability of IFN-γ to induce $p21^{waf1}$ mRNA expression correlates with its ability to inhibit cell growth and requires the presence of STAT1 [5]. Furthermore, STAT1 was shown in an electrophoretic mobility shift assay to be able to bind to oligonucleotide probes corresponding to the potential STAT-binding sites in the $p21^{waf1}$ promoter. Whether the $p21^{waf1}$ promoter is directly regulated by STAT1 remains to be determined, but these data strongly suggest that one way in which IFN-γ inhibits cell growth is through STAT1-dependent expression of $p21^{waf1}$. Other investigators have suggested that in addition to $p21^{waf1}$, the expression of another STAT1-regulated gene, IRF-1, may also contribute to IFN-γ-elicited growth arrest [8]. Sustained activation of STAT1 is also required for IFN-α inhibition of malignant melanoma cells [9].

Epidermal growth factor (EGF) and fibroblast growth factor (FGF), although generally considered mitogens,

will inhibit proliferation of many cell types in a STAT1-dependent manner. For example, EGF treatment of A431 fibroblasts, which unlike normal fibroblasts arrests growth in response to EGF, activates STAT1 [5, 10] and induces $p21^{waf1}$ expression [5]. Expression of a dominant-negative STAT1 in A431 cells reverses the anti-proliferative action of EGF, suggesting that STAT1 is necessary for growth arrest [10], though whether $p21^{waf1}$ levels are also reversed has not been examined. The growth of MDA468 breast carcinoma cells is also inhibited by EGF, and this is likewise associated with $p21^{waf1}$ induction; however, in these cells, EGF activates both STAT1 and STAT3, so whether one or both are involved in $p21^{waf1}$ transcription remains to be determined [11]. FGF-induced growth arrest of another breast cancer cell line, MCF-7, is similarly correlated with STAT1 activation and $p21^{waf1}$ [12]. The authors speculate that FGF may normally play a beneficial role by limiting the growth of malignant breast cancer cells and note that decreased levels of parenchymal FGF correlate with tumor progression. In contrast, the sustained STAT1 activation and $p21^{waf1}$ elevation in chrondrocytes due to expression of a constitutively active FGF receptor (TDII FGFR3) may be detrimental and responsible for the lethal condition, thanatophoric dysplasia type II dwarfism [13]. However, whether additional pathways activated by FGF3 may contribute to the observed growth inhibition observed in this disease has not been ruled out.

STAT3 and proliferation

The involvement of STAT3 in the growth arrest elicited by interleukin (IL)-6 in M1 myeloleukemia cells [14] or IL-10 in J774.1 macrophage cells [15] was suggested by reversal of the inhibition by dominant-negative STAT3 expression. Furthermore, the inhibitory action of IL-10 was shown to be mimicked by a STAT3-GyrB fusion protein which undergoes dimerization in the presence of the drug coumermycin A1 [15]. Examination of cell cycle protein expression has suggested that induction of the cell cycle $p19^{INK4d}$ may contribute to both IL-6- and IL-10-dependent growth arrest [14, 15]. The transcription of $p19^{INK4d}$ is likely to be directly regulated by STAT3 since induction is independent of de novo protein synthesis and a 1.6-kb $p19^{INK4d}$ promoter fragment, which contains two potential STAT-binding sites, responds to IL-10 in a luciferase-reporter assay [O'Farrell et al., unpublished data].

On the other hand, STAT3 activation is also associated with IL-3 stimulation of myeloid 32Dcl13 cells [16, 17], angiotensin II (Ang II)-induced vascular smooth muscle cell proliferation [18], and IL-6-driven growth of BAF/B03 cells. In the 32D system, v-src-mediated abrogation of the IL-3 dependence is accompanied by activation of STAT3 and its physical association with the v-src encoded protein [16]. The relevance of these findings to normal IL-3 signalling has been substantiated by the observation that IL-3 activates endogenous the c-src kinase, and that inducible expression of a dominant-negative c-src inhibits both STAT3 phosphorylation and IL-3-driven proliferation. The authors also conclude that Janus kinase (JAK)2, in contrast to previous studies [19], plays no role in IL-3-dependent proliferation because constitutive expression of a kinase-dead JAK2 in 32Dcl13 cells was found to have no effect on either STAT3 activation or cell growth. However, the constitutive expression of the dominant-negative JAK2 may have allowed selection of a population of cells adapted to growth in the presence of diminished levels of endogenous JAK2 activity. In another approach to assess the contribution of STAT activity, neutralizing anti-STAT antibodies were introduced into vascular smooth muscle cells (VSMCs) by electroporation [18]. The presence of these antibodies significantly reduced VSMC proliferation in response to Ang II but not platelet-derived growth factor (PDGF), suggesting that STAT1 and 3 are necessary for Ang-II- but not PDGF-stimulated growth. STAT3 activation has also been suggested to contribute to IL-6-dependent BAF/B03 cell growth through binding to the *cis*-regulatory region of the *c-myc* gene [20].

STAT5 and proliferation

STAT5 (STAT5 refers to both STAT5a and STAT5b isoforms) is activated by many receptors including those for IL-2, IL-3, or erythropoietin (EPO). Studies exploring the role that STAT5a/b may play in proliferation stimulated by these cytokines have made use of dominant-negative STAT constructs, receptor mutants lacking STAT5-interacting tyrosines and, more recently, STAT5a$-/-$ or b$-/-$ mice. Some investigators have concluded that STAT5 is not necessary for proliferation, but an alternate interpretation of the current data suggests that although STAT5a/b is not absolutely required for proliferation, it does contribute to the growth response.

The IL-2 receptor (IL-2R) consists of α, β, and γ_c subunits, the β subunit being responsible for interacting with and leading to the activation of STAT5. Early studies reported that truncation mutants of the IL-2Rβ chains lacking the STAT5 activation domain are still able to transduce IL-2-dependent proliferative signals in Ba/F3 cells [21]. However, closer examination of the data has revealed that the ability of the relevant mutant (H-4) to support IL-2-driven thymidine incorporation is reduced relative to the wild-type receptor by a statistically significant amount. Similarly, significant attenua-

tion was observed by other investigators [22], who also concluded that the STAT5 pathway acts in concert with other signalling pathways to maximize proliferation. Other evidence suggesting a proliferative function for STAT5 comes from the observation that wild-type replacement of a naturally occurring EPO receptor (EPOR) mutant, impaired for STAT5 activation, restored the ability of EPO to support TF-1 cell proliferation [23]. Directed substitution of the tyrosines in the human EPOR responsible for STAT5 activation also diminished the ability of the receptor to support EPO-dependent growth [24, 25], the effect being most evident at lower EPO concentrations [24]. At concentrations of EPO greater than 1 U/ml, receptors defective in the STAT5 pathway behaved like wild-type receptors [24, 26]. This result is not surprising since EPOR-associated, non-STAT5 pathways are sufficient to support considerable proliferation [27]. However, the relevance of STAT5 to EPO-driven growth is further strengthened by the recent observation that the synergism between insulin-like growth factor-1 (IGF-1) and EPO for proliferation of myeloid F-36P cells may be mediated through IGF-1 enhancement of STAT5 tyrosine phosphorylation [28].

A role for STAT5 in mediating proliferative responses to IL-3 has also been indicated. The IL-3 receptor consists of a ligand-specific subunit, and a signalling component referred to as β_c which is also utilized by the receptor complexes for IL-5 and granulocyte-macrophage-colony-stimulating factor (GM-CSF). Mutational analysis of β_c has suggested that none of the receptor tyrosines are necessary for STAT5 activation [29, 30], consistent with subsequent reports indicating that STAT5 can be activated by direct association with JAK2 [31, 32]. For this reason, the receptor tyrosine substitution approach to selectively abrogate STAT5 activation could not be applied to the study of STAT5 function in IL-3 signalling. As an alternative, dominant-interfering, C-terminally truncated STAT5a molecules were inducibly expressed in the IL-3-dependent Ba/F3 cell line. Expression of the dominant-negative STAT5 suppressed, though did not abolish, IL-3-driven growth, suggesting that the STAT5 pathway contributes to the proliferative response. The molecular basis of this impairment is not clear. The ease with which cells adapt and compensate for the growth retardation, after a short time in culture (A. L.-F. Mui, unpublished data), may be the reason why constitutive expression of a similarly truncated STAT5 in another IL-3-dependent cell resulted in no apparent growth defect [26]. In addition to the cytokine systems described above, a recent study has suggested that ligation of the T cell antigen receptor (TCR) also activates STAT5. Blocking the STAT5 pathway using dominant-interfering molecules inhibited TCR-driven proliferation in a manner independent of autocrine IL-2 production [33].

Caveats associated with the use of mutant receptors or dominant-interfering STATs to probe signalling pathways include the possibility that pathways other than the one of interest may be affected. One way to address this concern is to analyze mice with one or both of the STAT5 genes disrupted [34–36]. The phenotypes of these mice are discussed in detail in the contribution by D. E. Levy. Briefly, mice disrupted for STAT5a, originally characterized as a prolactin-activated factor, exhibit impaired mammary lobuloalveolar outgrowth during pregnancy [34], whereas disruption of STAT5b, the major isoform expressed in liver, results in defective growth-hormone-regulated liver gene expression [36]. The effects of STAT5a or STAT5b deletion on the function of cytokines previously shown by in vitro studies to utilize STAT5 are beginning to be reported. Macrophages from STAT5a $-/-$ mice are impaired in their response to GM-CSF [37], a defect reminiscent of the diminished proliferative response observed in vitro in the related IL-3-signalling system [29]. T cells from STAT5a $-/-$ mice fail to upregulate IL-2Rα in response to IL-2, a process previously shown to be STAT5 dependent [38, 39]. This suggests an indirect role for STAT5a in enhancing IL-2-induced T cell proliferation. However, the possibility of an additional intrinsic IL-2 response defect cannot be ruled out until T cells with equivalent high-affinity IL-2Rs from wild-type and STAT5a $-/-$ are compared. The double STAT5a and STAT5b targeted animals [35] possess mammary gland and liver developmental failures similar to the single disruptants. Deficiencies in T cell proliferation and IL-3/IL-5- and GM-CSF-driven in vitro colony formation are also observed. It will be interesting to characterize the mechanistic basis for these defects and determine whether they can be related to the in vitro studies performed with mutant receptors and dominant-negative STAT proteins.

STAT6 and proliferation

Initial studies describing the STAT6 $-/-$ phenotype [40–42] reported the loss of IL-4-dependent functions in T and B cells, including mitogenesis, T-helper (Th) cell differentiation, and immunoglobulin isotype switching. The diminished proliferative capacity was unexpected since earlier analysis of IL-4R-deletion mutants had suggested that although the membrane proximal region of the receptor responsible for phosphorylation of a protein called 4PS (IL-4 phosphorylated substrate, also IRS-2) was required for cell growth, the membrane distal domain responsible for STAT6 activation was dispensable [43–45]. An essential role for the 4PS protein in IL-4-stimulated mitogenesis is also consistent with earlier cell reconstitution experiments [46]. For these reasons, the decreased mitogenic response to IL-4

was speculated to be secondary to the failure of STAT6 $-/-$ mice to upregulate IL-4R numbers in response to IL-4. However, the IL-4R tyrosine necessary for 4PS phosphorylation is able to activate STAT6, albeit to a much lesser extent than the membrane distal tyrosines [46]. Furthermore, an IL-4R-expression-level-independent role for STAT6 in enhancing IL-4-dependent lymphocyte proliferation was recently shown by more detailed examination of cells from STAT6 $-/-$ animals [47]. Lymphocytes expressing equivalent amounts of IL-4R were isolated by flow cytometry from wild-type and STAT6 $-/-$ animals and tested for their proliferative response to IL-4. Despite expressing similar levels of IL-4R, cells from Stat6 $-/-$ mice were impaired in their ability to undergo IL-4-dependent growth and this appeared to be due to the inability of IL-4 to downregulate expression of the cell cycle inhibitor $p27^{KIP1}$ [47]. Disruption of IL-12-dependent proliferation in STAT4 $-/-$ lymphocytes [48, 49] appeared also to be, at least in part, due to a failure to downregulate $p27^{KIP1}$ [47]. IL-4 downregulation of $p27^{KIP1}$ in lymphocytes occurs post-transcriptionally, but how STAT6 regulates this process is not clear [47].

The role of STATs in differentiation

In addition to their roles in cell growth, STAT proteins also participate in differentiation and or functional maturation of many cell types. While STAT3 and STAT5 have been implicated in myeloid cell development and appear themselves to be regulated in a cell type or developmental manner, STAT4 and STAT6 are best characterized for their central role in Th cell differentiation.

STAT3 and differentiation

An essential role for STAT3 in IL-6-induced macrophage differentiation of myeloid M1 cells was first suggested by the inability of the IL-6R-signalling-subunit (gp130) mutants, lacking the YXXQ STAT3-binding motif, to generate growth arrest and differentiation [50]. Subsequently, dominant-negative forms of STAT3 were found to inhibit IL-6 induction of differentiation-associated markers such as Fcγ receptors, ferritin light chain and lysozyme in these cells [51]. Interestingly, the presence of dominant-negative STAT3 converted the action of IL-6 from a differentiation to proliferative agent [52], suggesting that IL-6 generates antagonistic signals and STAT3 activation is pivotal in determining whether cells differentiate or proliferate in response to this cytokine. IL-6-induced differentiation of B cells, as assessed by IgM secretion, is also STAT3 dependent [53]. Granulocyte-colony-stimulating factor (G-CSF)-induced neutrophil differentiation of LGM-1 cells could also be inhibited by expression of a dominant-negative STAT3 [54]. However, although both G-CSF-elicited neutrophilic morphological changes and myeloperoxidase (MPO) induction are abolished by phenylalanine replacement of the tyrosine in the G-CSFR responsible for STAT3 activation (i.e., Tyr703) [55], the dominant-negative STAT3 constructs only abrogated the morphological changes, without affecting MPO levels [54]. This suggests MPO induction is (i) not as sensitive to dominant-negative STAT3 expression, and low-level or residual wild-type STAT3 activation is sufficient for expression, (ii) regulated by STAT3 in a manner independent of its dimerization or DNA-binding ability, or (iii) regulated by a non-STAT3 pathway also emanating from Y703. However, ciliary neurotrophic factor (CNTF), another cytokine that utilizes gp130, clearly signals through STAT3 to induce the differentiation of neuroepithelial precursors into astrocytes [56, 57]. STAT3-dependent pathways also contribute to morphogenic processes. HGF-induced tubulogenesis [58], and leukemia inhibitory factor (LIF)-stimulated cardiac myocyte hypertrophy [59] can both be reversed by dominant-negative STAT3 expression.

Conversely, STAT3 activation can also suppress differentiation. For example, the ability of LIF to keep embryonic stem (ES) cells in a pluripotent state is abrogated by overexpression of a dominant-negative STAT3. Furthermore, the ability of retinoic acid treatment to overcome the effect of LIF and induce differentiation of ES cells is characterized by a rapid decline in the levels of tyrosine phosphorylated STAT3 [60]. Adenovirus-E1A-mediated inhibition of ES cell differentiation is likewise associated with a block in LIF-induced STAT3 DNA-binding activity [61]. GM-CSF inhibition of EPO-induced erythroid differentiation of the human leukemia line UT-7, on the other hand, correlates with the activation of STAT1 and STAT3 [62, 63] and this can be mimicked by overexpression of either STAT1 or STAT3 [64].

The dual roles for STAT3 in both induction and inhibition of differentiation probably reflect the contribution of other modulating signalling pathways, or the status of the cell being regulated. However, an additional possibility arises from the existence of two STAT3 splice variants, STAT3α and STAT3β. STAT3β, the short form, differs from the originally described STAT3α in lacking 55 C-terminal amino acids and having an additional 7 STAT3β-specific residues [65]. The two also differ in functional properties; for example, overexpression of STAT3β but not STAT3α results in constitutive transcriptional activity on an IL-6-responsive promoter [65], while STAT3α exhibits greater transcriptional activity in response to external stimuli

[66]. In some cells, STAT3β behaves in a dominant-negative manner towards STAT3α [67]. Although the levels of STAT3β mRNA predominate in most tissues, STAT3α transcripts could be detected in brain, lung, heart, ovary and spleen [66]. The significance of the observed distribution is not clear. Intriguingly, however, CD34+ hemopoietic progenitor cells and leukemic myeloid cell lines which differentiate with G-CSF treatment activate STAT3β in response to this cytokine, whereas leukemic cell lines refractory to differentiation activate both STAT3α and STAT3β [68].

STAT5 and differentiation

STAT5 has been implicated in differentiation in a variety of systems. A role for STAT5 in mammary gland development and milk protein expression was indicated by the increase in STAT5a/b transcripts and DNA-binding activity during late pregnancy, and their rapid decline during mammary gland involution [69, 70]. This hypothesis was subsequently proven by the mammary gland differentiation defects in STAT5a$-/-$ mice [34]. In addition, despite the established importance of STAT3 deactivation on ES cell differentiation, recent studies suggest that the concomitant upregulation of STAT5 mRNA and activity are also important during this process [60, 71]. Furthermore, a transcription factor complex designated differentiation-induced factor and involved in macrophage differentiation of promonocytic U937 cells [72] was found to contain STAT5 [73]. Similarly, the differentiation of human myeloid HL60 cells and chick myeloid progenitor cells correlated with enhanced STAT5 activation [74]. Apart from the milk protein genes expressed in mammary gland cells, the differentiation-related, molecular targets of STAT5 are not yet defined.

STAT5 has also been reported to be involved in thrombopoietin (TPO)-dependent maturation of megakaryocytes. Differentiation is usually accompanied by growth arrest in the G1 phase of the cell cycle and TPO treatment of the CMK megakaryoblastic leukemia cell line results in megakaryocytic differentiation and induction of the cell cycle inhibitor $p21^{CIP1}$. In general, whether the loss of growth arrest is a consequence or cause of differentiation is not clear; however, in the CMK cell system, ectopic expression of the $p21^{CIP1}$ cell cycle inhibitor is sufficient to induce differentiation [75]. TPO activates both STAT3 and STAT5, but only STAT5 was able to react with oligonucleotide probes corresponding to STAT-binding sites in the $p21^{CIP1}$ promoter. This suggests that one mechanism operating during TPO-directed megakaryocyte differentiation is STAT5-dependent induction of $p21^{CIP1}$, although the coordinate action of STAT3-regulated events may also contribute [76].

Studies making use of EPOR mutants and dominant-interfering STAT5 constructs to examine the role of STAT5 in EPO-dependent differentiation have generated puzzling results. Murine erythroleukemic SKT6 cells undergo hemoglobinization upon EPO stimulation and this response is abrogated by either mutation of the STAT5 interaction motif in the EPOR, or by expression of a dominant-negative STAT5 [77, 78]. EPORs lacking the ability to activate STAT5 are likewise impaired for differentiation of other erythroleukemia cell lines [79, 80]. These studies suggest STAT5 is involved in erythroid differentiation. However, other studies demonstrate that chimeric receptors, consisting of the extracellular domain of the EPOR and the intracellular domain of a receptor that normally inhibits erythroid differentiation (IL-3R), can also drive differentiation [81, 82]. Furthermore, the EPOR mutant that best reconstitutes definitive erythropoiesis in fetal liver cells derived from EPOR$-/-$ mice is not the one that is best able to activate STAT5 [83].

Recent reports provide a possible explanation for the apparent paradox by suggesting that although STAT5 is involved in erythroid maturation, high-level activation may be inhibitory. The endogenous EPOR in the human TF1 erythroleukemia cell line carries a mutation in the STAT5-binding site, which impairs its ability to activate STAT5, and EPO stimulation of these cells sustains only short-term proliferation before differentiation ensues. Introduction of a wild-type receptor into TF1 cells restores high-level STAT5 activation and this is accompanied by a switch from a predominantly differentiative to a proliferative response [23]. The correlation of high-level STAT5 activation with proliferation has been observed in other systems. Ligation of the c-ErbB receptor on erythroid progenitors is able to induce a STAT5-responsive reporter gene and sustain proliferation, while EPO treatment, which fails to stimulate reporter gene activation, results in erythroid differentiation [84]. More strikingly, the ability of chimeric receptor constructs to signal β-globin expression correlates not with their possession of EPOR domains, but rather with their support of only low-level STAT5 activation [85]. These data can be drawn together into a model in which a certain amount of STAT5 activation is required for differentiation, but above a critical threshold, genes are induced which are not compatible with differentiation. Expression- or activation-level-dependent biological outcomes for other transcription factors have previously been proposed [86]. A molecular basis for this threshold effect might be varying binding affinities of STAT-responsive elements in the promoters of different genes. Thus different levels of STAT activation may induce a different spectrum of genes. Regardless of the mechanism, a quantitative model of STAT5 action is consistent with other observations: the sup-

pression by IL-3 (a strong STAT5 activator) of EPO-dependent differentiation, and the ability of a tyrosine null EPOR (weak STAT5 activator) but not of a cytoplasmic-domain-truncated EPOR (no STAT5 activation) to induce β-globin expression [87]. This model can also accommodate the reported ability of an EPOR containing only one cytosolic tyrosine residue, Y479, to support proliferation and differentiation of primary erythroid progenitors [83]. Although Y479 is not primarily responsible for STAT5 activation, the Y479 EPOR would still activate low levels of STAT5 [24, 25]. This low-level STAT5 activation may act in combination with Y479-dependent pathways to support erythroid development. A challenge to this model, however, is the apparently normal erythroid development in STAT5a/b $-/-$ mice [35]. It will be of interest to examine whether EPO utilizes alternate STAT proteins in cells lacking STAT5.

In addition to the strength of activation, the choice of STAT5 isoform utilized can also determine the genes regulated and thus the overall biological consequence. That STAT5a and STAT5b have separate as well as redundant functions has best been shown by the phenotypes of the mice disrupted for the two genes [34–36]. However, in vitro studies have also pointed out instances of cytokine and cell-type-specific utilization of STAT5a or STAT5b. For example, GM-CSF activates STAT5b but not STAT5a in neutrophils [88] despite the expression of both. IFN-γ utilizes STAT5a in myeloid U937 cells and IFN-α activates STAT5b, but only in HeLa epithelial cells [73]. The mechanism by which the receptors for these cytokines are able to specifically select one of the STAT5 proteins, and the consequences of differential usage to biological function remain to be established.

Adding to the complexity of STAT5 signalling is the occurrence of naturally truncated STAT5 protein products. A carboxyl-truncated transcriptional splice variant of STAT5a that acts as a dominant-negative has been described [89], although neither tissue distribution nor the regulation of expression of this naturally occurring mutant has been reported. Both STAT5a and STAT5b are also subject to post-transcriptional processing to smaller, approximately 80-kDa isoforms [90, 91]. Whereas IL-3 stimulation of phenotypically more mature myeloid cell lines leads to tyrosine phosphorylation and activation of DNA-binding activity of full-length, $\sim$90-kDa STAT5 proteins, similar treatment of more immature cell types induces only the $\sim$80-kDa products [90]. Biochemical analysis of this phenomenon suggests the presence of a STAT5-specific protease in immature but not mature myeloid cells [91]. The IL-3-related cytokine, GM-CSF, also activates an 80-kDa STAT5 molecule in freshly isolated monocytes, and expression of this 80-kD protein likewise disappears upon differentiation into macrophages [92]. Murine macrophage lines representing various stages of macrophage differentiation also express either full-length or truncated STAT5 depending on their state of maturation [93]. The 80-kDa isoforms of STAT5 have also been reported in peritoneal macrophages [94] and Nb2 T cells [95]. It has not as yet been determined whether the smaller forms of STAT5 observed in these studies result from transcriptional or post-transcriptional processing. Regardless of how they are generated, the existence of these multiple forms of STAT5 may provide another mechanism by which cell- or maturation-stage-specific actions of STAT5 are controlled, since truncated STATs have different functional properties to the full-length molecule [29, 89, 96, 97]. Indeed, examination of IL-3-induced immediate-early gene expression confirmed that genes previously characterized in mature myeloid cell lines to be STAT5 regulated were not elevated in response to IL-3 in the immature myeloid lines expressing the 80-kDa isoform of STAT5 [91].

The role of STAT4 and STAT6 in differentiation

The contribution of the STAT pathway to differentiation has been most definitively shown for STAT4 and STAT6 in Th cell maturation. STAT4 and STAT6 are required for the IL-12- and IL-4-dependent development of Th1 [48, 49] and Th2 [40–42] cells, respectively, from a common precursor [98]. However, although IL-12 and IL-4 are commonly referred to as cytokines that 'drive' Th cell differentiation, they may function primarily to expand and enhance the differentiation of precursors already committed towards Th1 or Th2. In fact, GATA-3 expression may be the key regulator of differentiation, since ectopic expression of GATA-3 in naive T cells is sufficient to skew development towards Th2 and conversely to inhibit Th1 differentiation [86, 99–101]. Elegant studies using cells from mice deficient for both STAT4 and STAT6 have also suggested the existence of a STAT4-independent pathway for Th1 development [47]. Thus, although STAT4 and STAT6 are clearly involved in expression of phenotypic markers of Th1 (IFN-γ) [102] and Th2 (IL-4R) [40] cells, whether activation of either STAT is sufficient to directly induce differentiation requires further study. For example, examining whether activation of the recently described inducibly dimerizable STAT6 [103] alone is able to drive Th2 development will be of great interest. STAT6 is also required for CD23 expression and IgE switching in B cells [41, 42].

Role of STATs in apoptosis

Although proliferation involves progression through the cell cycle and cytokines can control this by modulating expression of cell cycle proteins, long-term cell growth also requires inhibition of apoptosis or programmed cell death. In some cytokine systems, this cell survival signal is provided by Ras [104], Akt [105], or other non-STAT [106, 107] pathways; however, STAT5 [107] and STAT3 [108, 109] have been shown to contribute to IL-2 and IL-6 anti-apoptotic signals, respectively. Apoptosis is additionally important as a means of regulating immune responses and also serves as an effector mechanism for the elimination of malignant or virally infected cells. Recently, several cytokines have been found to direct apoptosis through STAT1-dependent activation of the pro-apoptotic caspase cascade [110, 111].

STATs and protection from apoptosis

As described above, STAT5 has been implicated in IL-2-dependent proliferation. Further examination of this phenomenon has suggested that the STAT5 pathway does not regulate cell cycle progression. Instead, its major function appears to be suppression of apoptosis [107]. In contrast, STAT3 activation appears to contribute both proliferative [20] and cell survival signals [108] towards IL-6-dependent BAF/B03 cell growth. Analysis of gp130 mutants has shown that the tyrosine residue essential for activation of STAT3 is also necessary for prevention of apoptosis in BAF-B03 cells [108]. Studies performed in STAT3-deficient T cells have confirmed the STAT3 requirement in IL-6-mediated prevention of apoptosis [109]. The mechanism by which STAT5 and STAT3 activation inhibits apoptosis is not clear. Although induction of the anti-apoptotic protein Bcl-2 has been shown to correlate with STAT3 activation and cell survival in the IL-6/BAF-B03 system, Bcl-2 does not appear to be involved in either STAT5-dependent protection of IL-2-stimulated 32D cells [107], or in IL-6- and STAT3-dependent survival of primary T cells [109]. STAT3 activation may also be involved in the ability of an activated *ras* allele to protect rat intestinal epithelial cells from ultraviolet-irradiation- or mitomycin-C-induced apoptosis [112]. Protection of these epithelial cells correlated with Bcl-2 expression.

STATs and induction of apoptosis

IFN-γ-elicited epithelial and immune cell apoptosis is associated with elevation of caspase-1 transcripts in a JAK1- and STAT1-dependent mechanism [110]. Cells deficient for any of these proteins are resistant to IFN-γ-induced cell death. Similarly, the ability of EGF to induce apoptosis rather than mitogenesis in different cell lines correlates directly with the ability of EGF to activate STAT1. STAT1 is also important for maintaining basal level expression of caspase family members in serum-dependent epithelial cell lines. STAT1-null U3A cells could not be killed by tumor necrosis factor (TNF)-α treatment, but reconstitution of these cells with STAT1 restored basal caspase expression and sensitivity to TNF-α-induced apoptosis [111]. Thus, STAT1 activation appears to be important for transducing apoptotic signals. However, attempts to determine whether caspase gene transcription is a direct target of STAT1 has not yet been conclusive [110]. A pro-apoptotic role for STAT3 has also been suggested based on the ability of the C-terminally truncated STAT3β to attenuate MHC II cross-linking-induced Jurkat T cell apoptosis [113]. Further studies are needed to establish whether C-terminally truncated STAT3β does behave as a dominant-negative molecule in this system, and to identify the genes regulated by the postulated pro-apoptotic action of STAT3.

STAT involvement in oncogenic transformation

Disregulated proliferation, resistance to differentiation, or insensitivity to normal death signals can all lead to oncogenic cell transformation. Thus, the occurrence of constitutively activated STATs in cancer cells underscores the importance of the STAT pathway in regulating these cellular processes.

Cytokine-independent T cell lines established from human T cell lymphotropic virus (HTLV)-1 immortalized T cells contain constitutively tyrosine phosphorylated STAT3 and STAT5 which can bind DNA [114]. This activation is associated with constitutively active JAK3 expression and does not appear to be an artifact of adaptation to growth in tissue culture since similar findings were obtained from uncultured, ex vivo T cells from HTLV-1-seropositive patients with adult T cell leukemia/lymphoma [115]. A slowly migrating isoform of STAT3 was similarly reported to be constitutively activated in mycosis fungoides (MF), a low-grade cutaneous T cell lymphoma of unknown etiology [116]. However, in contrast to the HTLV-1 disease, STAT3 activation was not accompanied by JAK3 activation in MF tumor cells. Moreover, STAT3 phosphorylated constitutively on serine 727, a phosphorylation known to be important for enhancing the ability of STAT3 to bind DNA or stimulate transcription [117, 118], was observed in B cells from 100% of patients with chronic lymphocytic leukemia [119]. In addition, the CD5+ subset of normal B cells, while not transformed, differs from the CD5− majority by their susceptibility to oncogenic transformation and expression of a constitutively nuclear-activated STAT3 [120]. Activated

STAT1, 3, and 5 have also been observed in myeloid leukemia cells [121–124] by many investigators.

Cell lines transformed by the oncogenic tyrosine kinases *v-src* [125, 126], *v-abl* [127], or *bcr-abl* [123, 124, 127] have also been reported to express constitutively activated STAT6, STAT3, or STAT5. This, together with the findings that STAT3 physically associates with the v-src protein [125] and that STAT5 phosphorylation occurs only at the permissive temperature in cells expressing a temperature-sensitive Bcr-abl [124], suggests that the STATs could indeed be a direct substrate for oncogenic kinases. This raises the question as to whether STAT activation is necessary for, or is merely a consequence of transformation. Recently, however, analyses using dominant-interfering mutants of STAT proteins showed that STAT activation is directly involved in transformation. The ability of *v-src* to transform 3T3 fibroblasts is abrogated by co-expression of STAT3 dominant-negative mutants that either could not bind DNA, or lack the C-terminal tyrosine necessary for STAT dimerization or Ser727 [128]. Likewise, expression of a dominant-negative STAT3 lacking the C-terminal transactivation domain inhibited the ability of *v-eyk* to transform rat fibroblasts [129]. Whether the central role STATs play in *v-src*- and *v-eyk*-driven transformation will hold true for other models of oncogenesis, and the mechanism by which the STAT pathway contributes to this process remain to be determined.

Perspective

Understanding of the role STATs play in biological responses has progressed considerably from the early days of cataloguing which stimulus activates which STAT to identification of cellular responses and target genes regulated by each STAT in each system. A number of controversies remain regarding the nature of the role played by certain STATs in some cytokine systems. However, the many molecular tools and STAT knockout mice now available should help clarify these questions. Defining how STATs regulate, and how they themselves are regulated, will provide insight not only the mechanism by which cytokines and other STAT activators transduce signals during normal physiology, but also importantly, into the disregulation that occurs in disease.

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