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Introduction: STATs as essential intracellular mediators of cytokine responses

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Cytokines form a complex regulatory network affecting cell growth, survival, differentiation and activation.
They transmit their biological message by binding to specific cell surface receptors. Like the cytokines themselves, their receptors can be grouped into families with distinct structural features and common ways of intracellular signal transduction [1]. Among the rapid changes that occur in cells upon receptor-cytokine interaction are those determining expression levels of specific target genes. Therefore, the receptor-derived signal must be rapidly transmitted to the cell nucleus and stimulate or repress transcription factors. Frequently, the activity of transcription factors is regulated by phosphorylation and therefore involves one or more cytokine-regulated protein kinases and phosphatases. Such enzymes can be targeted by cytokine receptors through a highly variable number of intermediate steps. For example, activation of MAP family kinases which phosphorylate transcription factors participating in growth or stress responses requires adapter molecules, nucleotide exchange factors, small G proteins and at least two additional protein kinases [2]. At the other end of the spectrum, SMAD subunits of transforming-growth-factor-β-activated transcription factors are phosphorylated directly by the receptor kinase [3].
The discovery of signal transducers and activators of transcription, the STATs, resulted from studies on rapid gene induction in response to interferons (IFNs, for details see the contribution by C. Schindler and S. Brutsaert). Transcription commences very rapidly after IFN receptors are occupied by ligand and, therefore, a rapid induction mechanism involving a small number of steps between receptor and IFN-responsive transcription factors was proposed [4]. Following the identification, characterization, and molecular cloning of these proteins, their activation mechanism was uncovered. It confirmed a rapid track between receptor and cell nucleus, established by the direct binding of transcription factor subunits to IFN receptors, their phosphorylation by receptor-associated kinases, and their subsequent movement to the nucleus requiring a dimerization reaction as the only additional protein interaction. Thus, the regulated protein subunits of IFN-responsive transcription factors became STATs because of their dual role as signal transducers between receptor and nucleus and as transcription factors [5, 6].

STAT activation by receptor-associated Janus kinases (JAKs)

STATs were the first and remain the only transcription factors known to be regulated by tyrosine phosphorylation [7]. Every STAT contains a conserved tyrosine residue near the C terminus and this is the only tyrosine that becomes phosphorylated upon activation. The structural counterpart to the phosphorylated tyrosine is the STAT SH2 domain located almost immediately before (i.e. towards the N terminus) the phosphorylated tyrosine. With the single exception of STAT2, STAT SH2 domains bind to the phosphotyrosine of a homotypic STAT, thus causing the formation of homodimers. In some cases, the SH2 domains also bind to a tyrosine-phosphorylated heterotypic STAT and promote the formation of heterodimers. Dimer formation has two important mechanistic implications. It exposes an undefined nuclear translocation signal and it enables STATs to bind to their target DNA sequences, in most cases a small palindrome, designated the GAS element [8]. Besides its critical role in dimer formation, the importance of the STAT SH2 domain lies in its ability to mediate the interaction with cytokine receptors. These become tyrosine-phosphorylated upon ligand binding. In several cases, direct binding of STATs to some of these receptor phosphotyrosines has been docu-

mented [9, 10]. In others, the receptor interaction may be mediated by a receptor-associated molecule, but nevertheless requires the STAT SH2 domain.

Receptors belonging to the class I (hematopoietin receptor family) or class II (IFN receptor family) cytokine receptor families contain a short, usually proline-rich, membrane-proximal amino acid motif frequently designated box1/box2. This stretch of amino acids serves to associate the receptor permanently with JAKs (see contribution by T. C. Yeh and S. Pellegrini). Initially identified by a clone-by-homology approach, the JAK TYK2 was shown in a genetic experiment to rescue the IFN responsiveness of cells selected on the basis of a deficiency in IFN signal transduction [11]. This key experiment established the essential character of JAKs in cytokine responses. Another member of the JAK family, JAK2, was shown by biochemical techniques to associate with and mediate immediate phosphorylation events triggered by the activated growth hormone (GH) and erythropoietin (EPO) receptors [12, 13]. These initial observations stimulated a plethora of investigations into the role of JAKs and STATs in cytokine responses. At the end of the day, a JAK-STAT paradigm was established, according to which cytokine receptors employ permanently associated JAKs to phosphorylate receptor chains and thus create phosphotyrosines for the docking of STATs and other signaling molecules. Subsequently, receptor-associated or otherwise recruited STATs are also phosphorylated by the JAKs. In retrospect, work on the cellular IFN response made the most important contribution to the discovery of the JAK-STAT signaling path because it led in the Darnell laboratory to the initial identification and cloning of transcription factors composed of STAT subunits. Moreover, the ability of TYK2, JAK1, JAK2, STAT1, and STAT2 to reconstitute different complementation groups of in-vitro-mutagenized, IFN-unresponsive cell lines, established in the laboratories of Ian Kerr and George Stark, provided compelling genetic evidence and carved the linear JAK-STAT path into stone [5].

The mammalian JAK family consists of four members (JAK1–3, TYK2) and the STAT family of seven members, excluding alternatively spliced or processed forms (STAT1–4, STAT5a, STAT5b, STAT6). As a rule, cytokine receptors activate distinct JAK-STAT combinations, but in some cases receptors use the same combination. For example, the GH, EPO, interleukin (IL)-3 and granulocyte-macrophage-colony-stimulating factor receptors all use JAK2 to activate predominantly STAT5 isoforms. JAKs seem to be the STAT kinases in the overwhelming majority of situations, but not all STAT activation results from a JAK-STAT path. Receptor tyrosine kinases probably phosphorylate STATs in vivo without a JAK requirement [14] and some non-receptor tyrosine kinases, e.g., v-Src or v-Abl may directly phosphorylate STATs as well.

Transcriptional activation of STAT target genes

STAT target genes fall into a number of distinct categories and many target genes appear to be regulated only by some but not all STAT dimers. Consistently, the biological effects of individual STATs are quite heterogeneous when analyzed in STAT-deficient animals. Since all STAT dimers prefer to bind to very similar promoter sequences in vitro, the basis of their target gene specificity is not entirely understood and probably involves numerous interactions with other transcription factors (see contribution by T. Decker and P. Kovarik). Such interactions may at least in part be mediated by the two most N terminal STAT domains. By contrast, the transactivation function, i.e., the ability to stimulate a transcriptional response after binding to DNA, lies within the last 50 or so STAT amino acids. It is not at all clear how this domain works, but the current state of the art would suggest that the mechanism of transactivation differs between individual STATs. In line with this assumption, some, but probably not all STAT C domains can be modified by regulated serine phosphorylation. The implications of serine phosphorylation are generally poorly understood and there may be several serine kinases which act in different situations and with preference for certain STATs. In only one case, STAT1, can the serine phosphorylation of the C domain be clearly linked to enhanced transcription factor activity [15]. Mechanistically, this enhancing effect is poorly understood.

Like many other transcription factors, STATs bind to the transcriptional co-activators P300 and CBP and this interaction can be mediated by the STAT N or C domains. P300/CBP are found in multi-protein complexes containing several co-activators with histone acetylase activities. Very little is known about the role of STAT-recruited co-activator complexes in chromatin remodeling of STAT target genes, but with the current pace of chromatin research, first insights can be expected in the near future.

Inactivation of STATs

Cytokine responses, particularly those promoting inflammation, must be tightly controlled. Consistently, STAT activity in cytokine-treated cells is very transient. It is now clear that the down-regulation of STAT activity is not primarily caused by a degradation of signal transducers, but mostly due to active shut-off mechanisms. These act at several levels in the activation cascade (see contribution by D. Hilton). At the level of JAK kinases, SH2-domain-containing phosphatase-1 (SHP-1) can cause dephosphorylation and inactivation of kinase activity [16]. Additionally, members of a recently described family of cytokine-induced proteins,

variously called SOCS, CIS/JAB, or SSI, have the ability to interfere with JAK-STAT signaling [17–19]. At least in some cases this may occur through direct binding to and inhibition of JAKs. At the level of STATs it appears clear that a nuclear phosphatase removes the essential tyrosine phosphate [20]. Proteins that bind to STATs and inhibit their activity have also been described. In particular, the protein inhibitors of activated STATs (PIAS), a family with several members displaying preference for individual STATs, have recently received widespread attention [21]. The relative contributions of the different signal inhibition mechanisms in various situations remains to be determined.

Participation of STATs in biological responses

Each STAT gene has been individually deleted from the mouse genome by homologous recombination (see contribution by D. Levy). Thus, the overt abnormalities of STAT-deficient organisms are known. Despite activation of some STATs by a large number of cytokine receptors (e.g., STATs 1, 3, and 5), the most striking phenotypes result from defects in a very limited number of cytokine responses. For example STAT1 is activated by IFN, IL-10, epidermal growth factor, platelet-derived growth factor, GH and other cytokines, but the only readily detectable phenotype of STAT1-deficient mice is a complete lack of natural immunity due to absent responsiveness to IFN [22, 23]. Similarly, targeted disruption of the STAT2, 4 and 6 genes primarily affects the activities, respectively, of type I IFN, IL-12 and IL-4/-13 [C. Schindler, personal communication; 24–27]. The phenotype of STAT5-deficient mice is more complex and affects mammary gland development, fertility, gene induction by GH, and T cell proliferation [28–30]. STAT3 deficiency causes lethality during embryonic development [31]. Recent reports with mice containing cell-lineage-specific STAT3 gene disruptions indicate a requirement for this family member during IL-6 and IL-10 responses [32, 33].

The undisputed power of gene-targeting experiments in revealing in vivo activity of the STATs has limitations where ontogenetic adaptation to life without a particular STAT occurs and STAT activity is being compensated for by other proteins (e.g., other STAT family members). Moreover, since STATs are essential components of both innate and adaptive immunity, phenotypes may be revealed only in particular pathologic situations. As outlined in the contribution of A. Mui, several cell biological activities of STATs can be demonstrated by a variety of approaches such as the mutation of STAT receptor binding sites or the expression of gain-of-function as well as loss-of-function alleles. These studies point towards a potential of STATs to influence growth, differentiation, and apoptosis of cells. Depending on the cellular system and the particular STAT under study, growth, differentiation, or apoptosis can be promoted or inhibited. Thus, one of the major questions remaining to be answered is the role played by STATs at different levels of cellular growth control in vivo. This questions extends to cellular transformation. In this regard, STAT1 was linked to tumor surveillance in mice [34] and STAT 3 shown in certain conditions to promote cellular transformation by viral oncogenes [35].

The evolution of STATs

The prominent role of STATs in the mammalian immune system might be taken to indicate that these proteins are a relatively modern 'invention' of evolution. However, this notion was first challenged by the identification of a *Drosophila* STAT and its role in both early and late stages of fly development [36, 37] (see contribution by C. Dearolf). Strikingly, STATs were also found in the slime mold *Dictyostelium discoideum*, an organism with both uni- and multi-cellular developmental stages [38]. Thus, STATs are found in even the most primitive multi-cellular organisms and their appearance coincides with the initial occurrence of phosphotyrosine-SH2 domain interactions at the metazoan boundary of evolution [39]. Besides the evolution of mammalian STATs from a single primordial gene, the functional radiation of this protein family is of particular interest. What was the original role of STATs in primitive metazoan organisms and when were they first connected to immune responses? With regard to the latter question, a STAT homologue in the fly *Anopheles gambiae* was recently linked to the infection-mediated induction of antibacterial peptides [40].

Concluding remarks

Apart from the fascinating topic of STAT evolution and their original function in invertebrates, the following series of reviews tries to follow STATs through a cytokine response. The molecular events leading to activation by JAKs, dimerization, nuclear translocation, and transcriptional activity are described, as are the biological consequences of STAT activity in cells and animals. The circle is completed by a summary of the events needed to reset the STAT-dependent signaling system by deactivation of the active components.

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