Review

Interleukin-7 Receptor Signaling Network: An Integrated Systems Perspective

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Interleukin-7 (IL-7) is an essential cytokine for the development and homeostatic maintenance of T and B lymphocytes. Binding of IL-7 to its cognate receptor, the IL-7 receptor (IL-7R), activates multiple pathways that regulate lymphocyte survival, glucose uptake, proliferation and differentiation. There has been much interest in understanding how IL-7 receptor signaling is modulated at multiple interconnected network levels. This review examines how the strength of the signal through the IL-7 receptor is modulated in T and B cells, including the use of shared receptor components, signaling crosstalk, shared interaction domains, feedback loops, integrated gene regulation, multimerization and ligand competition. We discuss how these network control mechanisms could integrate to govern the properties of IL-7R signaling in lymphocytes in health and disease. Analysis of IL-7 receptor signaling at a network level in a systematic manner will allow for a comprehensive approach to understanding the impact of multiple signaling pathways on lymphocyte biology. *Cellular & Molecular Immunology*. 2008;5(2):79-89.

Introduction

Interleukin-7 (IL-7) is a vital cytokine for the development and survival of T and B cells (1). IL-7 is produced primarily by fibroblastic reticular cells in the T cell zone in lymphoid organs (2) and binds to the IL-7 receptor complex, a heterodimer consisting of the IL-7R alpha chain (IL-7Rα) and the common gamma (γc) chain (3). While IL-7 shares pro-survival and proliferative capacities with related interleukin family members, it also plays non-redundant roles in T and B cell development and homeostasis (1). IL-7Rα is also expressed on dendritic cells (DCs) and monocytes, suggesting a possible role for IL-7 in multiple hematopoietic lineages (4). Major signaling cascades activated by the binding of IL-7 to IL-7R include the Jak-Stat and PI3K-Akt pathways (5). In lymphocytes, IL-7R signaling results in survival, proliferation and differentiation, depending on the developmental stage of the lymphocyte. In dendritic cells,

IL-7R signaling has an immunomodulatory role, especially in the context of thymic stromal lymphopoietin (TSLP), which also signals through the IL-7Rα in a heterodimeric complex with the TSLP receptor (TSLPR). Both IL-7 and IL-7Rα knockout mice have been studied. IL-7 knockout mice are deficient in T cells, B cells, NK cells, and NKT cells as well as intra-epithelial lymphocytes (6). Similarly, mice lacking IL-7Rα have a similar but more severe phenotype than IL-7 knockout mice (7), possibly because TSLP signaling is also abrogated in the IL-7Rα knockout mice. Binding of TSLP to the IL-7Rα:TSLPR complex can activate at least some of the signaling cascades normally activated by IL-7 but it also triggers some unique signaling pathways involving the Tec kinases (8, 9). IL-7 is considered to be present in limiting amounts in the body and competition for IL-7 maintains mature T cell homeostasis by limiting the size of the surviving T cell population (10). Consequently, IL-7Rα is under tight transcriptional control, and the cell type and stage-specific regulation of IL-7Rα ensures optimal utilization of limited cytokine stores. Negative feedback to IL-7Rα transcription, triggered by IL-7R signaling, is believed to maximize the size of the population in the presence of limiting amounts of IL-7 (11). Conversely, other network control mechanisms such as positive feedback loops that amplify the IL-7R signal have also been observed. The application of network approaches to the study of IL-7R signaling will lead to an integrated picture of the mechanisms contributing to this complex regulatory control.

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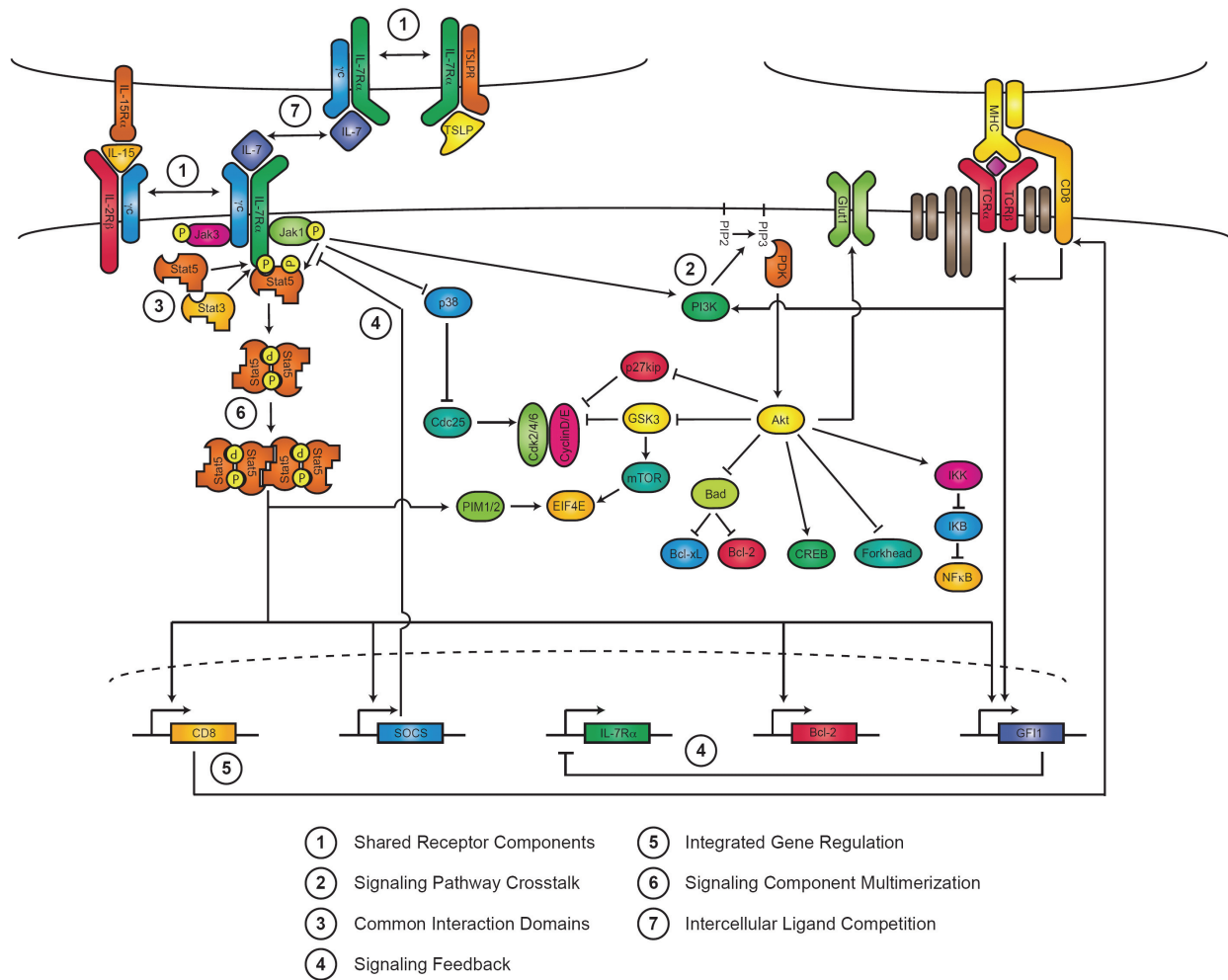
Figure 1. Control mechanisms in the IL-7R signaling network. A schematic diagram of the IL-7R signaling network and its connectivity with interacting signaling networks, including TSLP, IL-15 and the TCR. Important 'network motifs' of signal control are labeled with circled numbers.

In recent years, the analysis of cell signaling networks using quantitative systems biology approaches has revealed emergent phenomena that are not obvious based on the study of the individual network components (12). Further, it has yielded a molecular understanding of previously observed phenomena at the level of the cell, such as cellular responses occurring only over a narrow range of ligand stimulation, and at the level of the entire organism, such as tissue homeostasis. Of particular relevance to the biology of IL-7 are lymphocyte differentiation checkpoints at various stages of development and the regulation of lymphocyte population size at the level of the organism. It is becoming increasingly clear that the IL-7R signaling network is regulated at multiple hierarchical levels in its signaling pathways. What is unclear is the relative contribution of the various arms of the IL-7R regulatory network in controlling IL-7R signaling efficacy and the IL-7R signaling network behavior as a whole in stimulating survival, proliferation and differentiation throughout lymphocyte development.

In this review, we present an analysis of the IL-7R signaling network and network behavior from an integrated systems perspective. A scheme of the IL-7R signaling network and its connectivity with other signaling networks in T cells is depicted in Figure 1. We have highlighted the occurrence of the following mechanisms of signaling control: shared receptor components, signaling pathway crosstalk, common interaction domains, feedback regulation, integrated gene regulation, multimerization and ligand competition. In subsequent figures (Figures 2-8), we will illustrate the potential of each 'network motif' to affect the properties of the IL-7R signaling network including the strength and duration of IL-7R signaling. Because the quantitative properties of many of the system interactions are poorly characterized at present, we employ idealized models with assumed parameters in presenting these illustrations. We find that the interacting network components have the potential to affect the biology of the IL-7R signaling network in important but non-intuitive ways. We discuss the
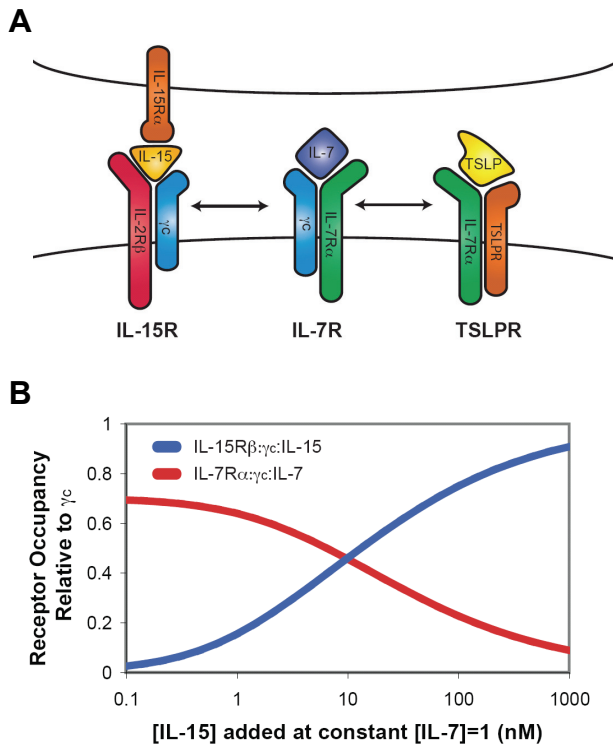
Figure 2. Influence of shared receptor components on signaling. (A) Receptor component chains shared among IL-7R, IL-15R and TSLPR complexes. IL-7R and TSLPR share the IL-7Rα chain, while IL-15R and IL-7R share the common γc. (B) Example of competition between IL-7 and IL-15 for the γc, assuming limiting levels of γc chain and that IL-7 binds with 10-fold greater affinity than IL-15 to its respective receptor. Even at constant levels of IL-7, the number of signaling competent IL-7:IL-7Rα complexes decreases with increasing levels of IL-15 as IL-15:IL-15Rβ sequesters available γc.

implications of these IL-7R signaling 'network motifs' on lymphocyte biology in health and disease. Since we have used idealized models, the predictions presented in this review are best viewed as hypothetical system properties that will require confirmation using quantitative experimental and analytical approaches. Model details associated with each figure are outlined in supplementary material for interested readers. (http://www.cmi.ustc.edu.cn/5/2/)

Shared receptor components

Several cytokine receptors are multimeric complexes made up of two or more different component proteins, which are often shared between multiple cytokine receptors. This can impact the relative availability of each receptor component, thereby limiting the extent of cytokine receptor signaling. Common examples of shared cytokine receptor components include sharing of the γc between the receptors for IL-2, -4, -7, -9, -15 and -21, the sharing of a common β chain between the receptors for IL-3, IL-5 and GM-CSF (13), and the

sharing of the β chain between IL-2 and IL-15 receptor complexes (14). In the case of the IL-7R complex, both receptor subunits are components of other receptors. As discussed above, the γc chain is shared with five other cytokine receptors, all of which regulate T cell growth and differentiation. As signaling specificity through the γc cytokine receptors is largely controlled by expression of the cytokine-specific α chains (as well as the β chain for IL-2 and IL-15), these subunits are kept under tight transcriptional control throughout development. The IL-7Rα chain can also form a heterodimeric receptor complex on binding to the TSLPR, which is homologous to the γc chain (15). IL-7Rα and TSLPR are co-expressed on T cells, pre-B cells and DCs (4). The ligand for the IL-7Rα:TSLPR complex is TSLP, which is a cytokine that is homologous to IL-7, and is produced by cells of epithelial origin in the thymus, lung, gut and skin (9). The presence of shared receptor components among cytokines can result in competition for the shared components among their respective ligands.

The association between the γc chain and IL-7Rα is required for the generation of a functional receptor complex and is mediated either by the binding of IL-7 or the activation status of T cells (16). The presence of competing cytokines can sequester IL-7 receptor components and adversely impact the ability to form functional IL-7R signaling complexes. For instance, IL-7 and TSLP can compete for IL-7Rα. Similarly, IL-7 and IL-15 compete for the γc chain (Figure 2A). One potential repercussion of shared receptor components among cytokines is an upper limit on the total amount of cytokine-specific signaling that a cell can receive in response to multiple cytokines. For instance, IL-15 can compete for the shared γc component when its expression is limiting (17). An example of the quantitative effects of this sharing is shown in Figure 2B, which illustrates how the number of IL-7:IL-7R complexes may decrease on exposure to increasing levels of IL-15 in the presence of a constant level of IL-7. However, the net signal strength down any given pathway may depend on the extent to which the γc chain is complexed with IL-7Rα or IL-15Rβ. The use of shared receptor components also partly helps explain the overlap in many cytokine receptor signaling pathways. As discussed in subsequent sections, this pleiotropy can also lead to competition for intracellular binding partners and downstream signaling effectors that can affect the propagation of signals from a particular receptor when multiple cytokines are present.

Aberrant receptor competition may be related to pathological outcomes. For instance, atopic dermatitis and asthma involve pathological Th2 differentiation induced by dendritic cells primed with TSLP (18). The effects of TSLP on T cells are largely mediated by modulating the function of DCs (19). IL-7 can also modulate DC function. For example, IL-7 is produced by inflamed synoviocytes in rheumatoid arthritis and it induces cell contact-dependent Th1 cytokine production in cocultures of synovial T cells and monocytes (20). Interestingly, skin keratinocytes have been shown to produce both IL-7 and TSLP, especially on exposure to
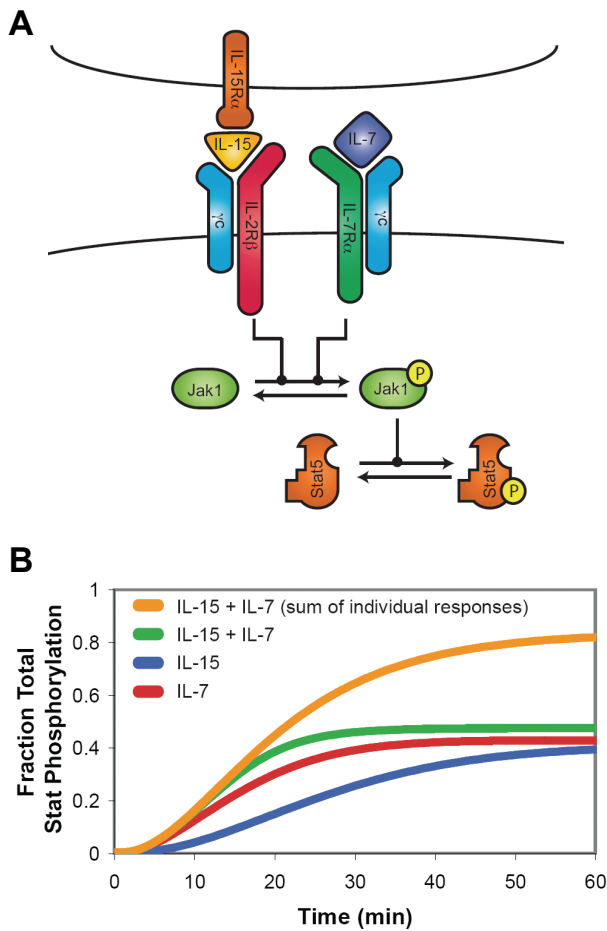
Figure 3. Influence of signaling pathway crosstalk on signaling. (A) Signaling through the common Jak-Stat pathway downstream of IL-7Rα and IL-15R. (B) Example of the anticipated kinetics of Stat5 phosphorylation after stimulation with either IL-7 or IL-15 or both. Here we assume equal IL-7 and IL-15 receptor numbers and ligand concentration but that IL-7 binds to its receptor with 10-fold greater affinity than IL-15, as well as first order Stat dephospho rylation. In this case, the combined IL-7 and IL-15 signal gives more rapid Stat5 phosphorylation than each of the cytokines alone, but a less than additive level of phosphorylation due to limiting amounts of Stat5. The combined signaling would approach the predicted additive signal upon increasing downstream Stat5 target expression.

certain pathogens (21, 22). The competing effects of TSLP and IL-7 on dendritic cells may therefore influence their ability to bias Th2 cell differentiation of CD4^{+} T cells that they activate. Further, we speculate that the possible ability of IL-7 to modulate the effects of TSLP by ligand competition may open new therapeutic avenues for asthma or atopic dermatitis.

Shared downstream signaling components

Many of the main signaling components of the IL-7R signaling pathway, including both positive and negative

regulators of cell proliferation and survival, are shared with other cytokine receptors. During such an interconnected signaling network response, multiple input cues work together through a small set of signaling network effectors that propagate down and spread to a number of downstream targets. Competition for shared signaling mediators can result in a hierarchy of responses controlled at the level of abundance and relative binding affinities to upstream regulators of the response.

The cross-specificity in Jak and Stat activation is one of the major modes of cytokine signaling crosstalk. The Jak kinase family is comprised of four members: Jak1, Jak2, Jak3 and Tyk2, each of which is found to be associated with multiple cytokine receptors (23). For instance, Jak1 is associated with the α subunits of γc cytokines such as IL-7Rα and IL-4Rα. Jak3 is associated with the γc chain (24, 25). Cytokine binding stimulates the trans-phosphorylation of receptor associated Jak kinases, which in turn phosphorylate tyrosine residues on the receptors themselves. The receptor phosphotyrosines serve as docking sites for SH2 domain proteins including the Stat family of transcription factors which are activated by Jak-mediated phosphorylation. Signaling crosstalk due to shared Jak kinases likely underlies many of the redundant signaling activities observed among interleukin family pro-survival cytokines. Cytokines, as well as many other growth factors, activate overlapping subsets of the seven Stat family members (Stat1, 2, 3, 4, 5a, 5b, and 6) (23). Thus, phosphorylation of multiple Stats by the Jak kinases also results in considerable crosstalk (26). Yet another point of crosstalk exists in the induction of suppressor of cytokine signaling (SOCS) family members. The SOCS proteins include eight family members (SOCS1-7 and CIS), each of which can inhibit signaling induced by multiple cytokines and growth factors by several mechanisms, including binding the Jak catalytic site, occupying the receptor Stat docking site, and targeting signaling proteins for degradation (27). SOCS1 is the major SOCS family protein involved in IL-7R signaling regulation and is induced by other cytokines, especially IFN-γ. Another major point of signaling crosstalk with the IL-7R signaling network is the PI3K-Akt pathway which is involved in a number of signal transduction networks that regulate cell survival (28). Each level of cross-specificity in these pathways, at the level of Jaks, Stats, SOCSs and PI3K-Akt targets, makes it harder to assign protein-specific effects and to deconvolve multi-cytokine responses.

Currently little is known about how pathways signaled by IL-7 are quantitatively regulated, especially in the context of concurrent signals derived from other cytokines or the T cell receptor (TCR). When signaling originates from multiple cytokines, it is likely that limiting amounts of common downstream targets can result in less than additive activation profiles. This phenomenon is illustrated in Figure 3, which examines the combined activation of the Jak1-Stat5 pathway by IL-15 and IL-7 in the presence of limiting Stat5. When both cytokine receptors are simultaneously ligated, the combined cytokine signals result in an increase in the rate of the response but little increase in the magnitude of the
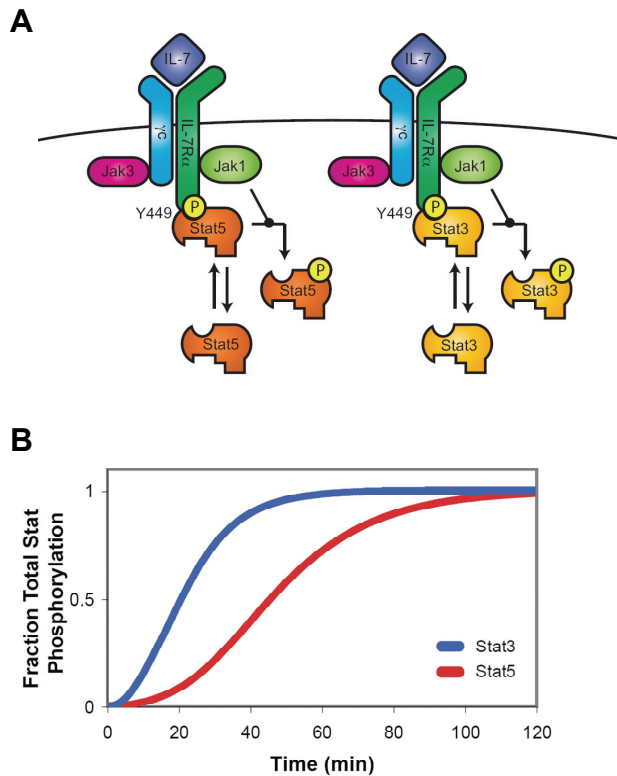
several distinct signaling proteins. Thus, many kinases have numerous substrates, and signaling scaffold proteins can recruit several different signaling mediators to the same domain. Yet, cytokines often predominantly activate only a small subset of these signaling mediators. Simultaneous stimulation with multiple cytokines which share overlapping downstream partners can also influence the strength and dynamics of the signal. Multiple cytokines can compete for the same binding site on a limiting number of receptors. Alternatively, the presence of the same signaling domain on multiple receptors can lead to competition for limiting downstream signaling mediators. Response specificity, timing and prioritization for pathway activation is thus dictated by the relative abundance and binding strength of interacting signaling proteins.

Several conserved interaction domains are found in the intracellular domain of the IL-7Rα chain. The cytoplasmic tail of IL-7Rα has two regions, Box1 and Y449, which are thought to be of particular importance for signal propagation regulating survival, proliferation and thymocyte development. Box1 is an eight amino acid membrane proximal motif that binds Jak1 and is found in all type I cytokine receptors. Y449 is one of three tyrosines in IL-7Rα, which is conserved between humans and mice, and recruits SH2 domain-containing Stat family members when it is phosphorylated by receptor-associated Jak1. Although Stat5 is the major Stat recruited to the Y449 site on IL-7R signaling, SH2 domain homology with other Stat family members could lead to competition among the Stats for binding to the Y449 site (Figure 4A). In particular, Stat1, 3 and 5 have been shown to be activated by IL-7R signaling (26, 31). However, a mutation at the Y449 site does not completely abrogate Stat1 and Stat3 signaling (31), suggesting additional routes for their activation by IL-7. Additional phosphotyrosine binding proteins like the Shc adaptor protein and insulin receptor substrate proteins may also compete with Stat5 for binding to the Y449 site (32). Competition at the Y449 site affecting Stat5 access could alter the timing and magnitude of Stat signaling. A hypothetical example of such competition between Stat5 and Stat3 is shown in Figure 4B. We have assumed that the Stat5:pY449 interaction is 10-fold stronger than the Stat3:pY449 binding and that the rates of Jak-mediated phosphorylation are equal for both Stats. The resulting delay in the kinetics of Stat3 phosphorylation, given limiting amounts of pY449 binding sites, is illustrated. Conversely, the presence of phosphotyrosines on other proteins that can also bind the SH2 domain of Stat5 could sequester Stat5 and hinder its binding to IL-7Rα. It has also been proposed that a second survival signal originating from the Y449 site arises from the recruitment of PI3K (33). The difference in binding kinetics of PI3K and Stat5 to the Y449 site could regulate the extent and timing of the signal through these two pathways, which may influence the downstream integration of survival signals. Quantitative experiments of signal dynamics under varying IL-7Rα, PI3K or Stat5 levels will be needed to determine how competition for binding sites impacts propagation of survival signals in lymphocytes.

Figure 4. Influence of conserved binding domains on signaling. (A) Competition between the SH2 domains of Stat3 and Stat5 for binding the phosphorylated Y449 residue in the IL-7Rα chain. (B) Hypothetical differences in the kinetics of activation of Stat5 and Stat3, which bind the same site (pY449) on the IL-7Rα receptor, with different affinities. Here we assume that the affinity of the pY449 site on IL-7Rα for Stat5 is 10 fold higher than that for Stat3, and that Stat3 and Stat5 phosphorylation rates by Jak1 are equal.

response over individual cytokine treatments. For simplicity, we have treated IL-15 as a soluble ligand like IL-7 in this example. However, it is now established that IL-15 is primarily bound to IL-15Rα by IL-15 producing cells *in vivo* (29). This is reminiscent of the biological activities of surface-tethered growth factors, which induce sustained signals as they prevent the internalization of the receptor complex (30). The effects of possible differences in receptor internalization rates of cytokines like IL-7, which is soluble, and IL-15, which is presented by IL-15Rα, have not been investigated. These differences could impact the dynamics of downstream signal competition. Well-designed quantitative experiments at the network level will be required to develop strategies for therapeutic manipulation of the network due to complex interconnected effects of multiple pathway activation.

Common interaction domains

The vast inter-connectivity of signaling networks is largely a result of overlapping binding specificities of multiple proteins for the same target motif, which often occurs in
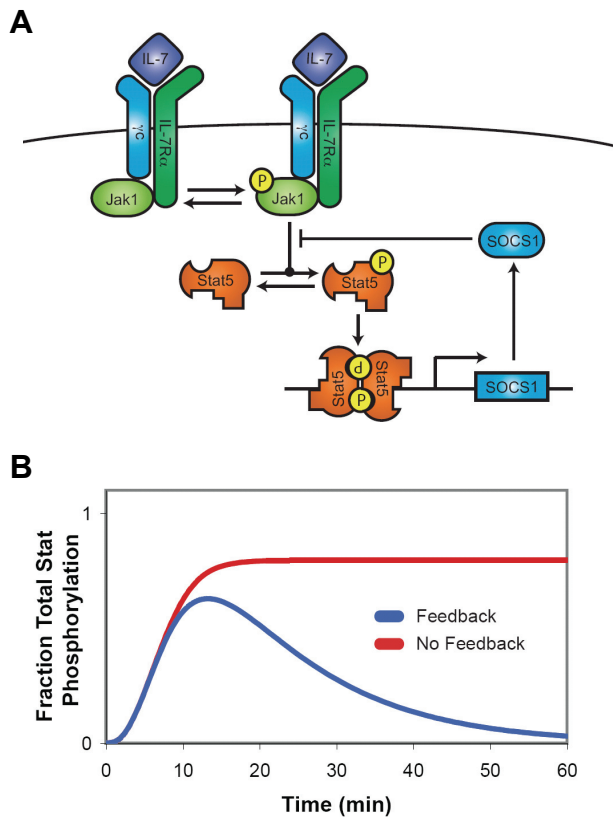
A

B

Figure 5. Influence of feedback control on signaling. (A) Negative feedback in IL-7 mediated Jak-Stat signaling, wherein activated Stat5 induces upregulation of SOCS1, which in turn inhibits Stat5 activation by Jak1. (B) Example of the kinetics of Stat phosphorylation in the presence and absence of negative feedback *via* SOCS1 upregulation and binding to activated Jak1, assuming first order Stat dephosphorylation. In the absence of feedback, the signal perpetuates, whereas SOCS1 upregulation leads to signal dissipation.

Signaling feedback control

Feedback loops comprise key mechanisms by which signal inhibition and propagation is controlled within cells. Signaling through a receptor may lead to signal inhibition *via* receptor internalization, induction of inhibitory phosphatases or transcriptional changes in receptor or regulator expression. Likewise, positive signaling feedback can be generated by inducing transcription of the receptor or its positive regulators or by autocrine secretion of a stimulatory ligand. Signaling feedback control is an important regulatory process in IL-7R signaling that allows avoidance of pathway saturation, establishment of signaling thresholds and fine tuning of the signal at an optimal level for cell survival. Understanding the balance of positive and negative feedback loops will be essential for a complete understanding of cytokine responses.

Negative feedback loops are particularly important in the regulation of IL-7Rα expression. Receptor ligation leads to

endocytic loss of the receptor from the surface, contributing to signal attenuation. In addition to receptor loss from internalization, several γc cytokines, including IL-7, activate both negative and positive feedback loops to modulate receptor mRNA expression. In CD8^{+} T cells, downregulation is mediated by the transcriptional repressor Gfi1, which is upregulated upon IL-7R signaling, as well as signaling by other interleukin family members (11). A second transcriptionally mediated negative feedback loop involves upregulation of SOCS1 expression upon cytokine signaling (Figure 5). SOCS1 can directly inhibit Jaks by acting as a pseudosubstrate through its kinase inhibitory region, as well as by ubiquitin-mediated degradation of the signaling complex itself (27). In addition, cytokine-independent regulation of SOCS1 also plays critical roles in regulating signaling by IL-7 and other γc cytokines throughout development. For example, SOCS1 is expressed at high level in DP cells during thymic development to prevent IL-7R signaling and possible aberrant positive selection (34). SOCS1 knockout mice show spontaneous activation of lymphocytes even in a pathogen free environment (35). A number of negative regulators of the Stats have also been identified such as the PIAS family of proteins (36). However, the relative contribution of these mechanisms of signal feedback inhibition to the overall control of IL-7R signal attenuation is yet to be elucidated.

IL-7R signaling also elicits positive feedback loops which contribute to signal amplification and sharp response thresholds. In developing B cells, IL-7R signaling causes upregulation of the transcription factors EBF and E2A, which in turn upregulate IL-7Rα, leading to a self-sustaining positive feedback loop (37). Feedback loops in IL-7R signaling play a critical role in B cell development by maintaining B cell lineage commitment among differentiating common lymphoid progenitors. In addition, sustained IL-7R signaling is necessary for survival of pro-B cells (38). EBF and E2A coordinately regulate the initiation of the B cell gene expression program as well as rearrangement of the immunoglobulin heavy chain loci (36). The signal feedback loops between these proteins ensure normal development of B cell precursors through various checkpoints in B cell development.

It was recently shown that in macrophages, Tat protein produced by the human immunodeficiency virus (HIV) can cause upregulation of IL-7Rα and increase IL-7R signaling. Increased signaling in turn promotes early infection events including viral entry, and ultimately efficient viral production (39). Interestingly, the effect of HIV Tat protein on CD8^{+} T cells was the opposite of that seen in macrophages, where it instead decreased IL-7Rα expression, inhibiting cell survival signaling (40). A detailed analysis of the complex, cell-specific feedback mechanisms will help better understand how the IL-7R signaling network is exploited by pathogens.

Integrated gene regulation

Interaction between signaling pathways at the gene regulatory level gives rise to a coordinated response and synergy in outcomes. Survival, activation and proliferation
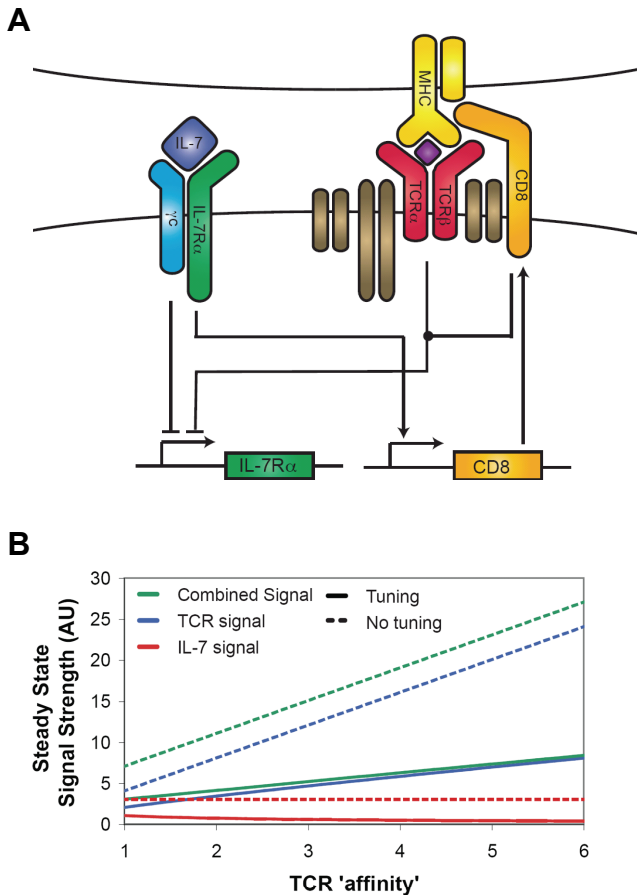
A

B

Figure 6. Influence of integrated gene regulation on signaling. (A) Co-regulation of TCR and IL-7R induced signaling according to the CD8 coreceptor tuning model. IL-7 transcriptionally increases CD8α expression to promote TCR-self peptide-MHC engagement, but TCR signaling impairs IL-7R signaling. (B) The predicted effects of coreceptor tuning are shown by plotting how TCR and IL-7R signals would be expected to vary as a function of TCR input signal strength ('affinity' for spMHC) with constant IL-7 input signal, either with or without TCR mediated IL-7R signal inhibition as described by the coreceptor tuning model. In the absence of tuning, TCR signaling is enhanced by IL-7 signaling and increases with the TCR input strength, whereas negative feedback to IL-7 dampens the TCR signaling induced by high 'affinity' ligands.

programs that are driven by antigen-receptor signaling and various γc cytokines are characteristic of lymphocytes. Microarray profiling has allowed for the high-throughput querying of gene expression programs regulated by cytokine and TCR signaling and their relationships with a variety of biological processes. High levels of overlap have been observed amongst the several hundred genes regulated by IL-2, IL-7 and IL-15. However, approximately 73% of these genes are also regulated by TCR signaling, and less than 20% of genes are unique to cytokine stimulation (41, 42). SOCS1 and Gfi1 are genes that are known to be transcriptionally induced during T cell activation as well as by pro-survival cytokine signaling. SOCS1 and Gfi1 inhibit the IL-7R

signaling pathway at the post-translational and transcriptional levels respectively. IL-7Rα itself is transcriptionally down-regulated by antigen-receptor signaling. In fact, it has been suggested that there is a greater overlap among TCR and interleukin-induced genes than amongst the genes induced by interleukin family members themselves (41). This strongly suggests that gaining a complete understanding of IL-7 induced signaling will require consideration of how related cytokines and TCR signaling influence the transcriptional network response.

As a specific example of the impact of interacting gene regulatory control, we have illustrated the phenomenon of coreceptor tuning which allows CD8⁺ T cells to maintain their antigen-receptor signaling at levels just below the threshold of autoimmunity (Figure 6A) (43). The levels of CD8 are a critical determinant of the responsiveness of a T cell to self-peptide MHC (spMHC) as CD8 promotes the kinetics of binding of TCR to a spMHC complex (44). Interestingly, CD8α is transcriptionally induced by IL-7R signaling while at the same time, IL-7R signals are inhibited by spMHC-induced TCR signal transduction. This leads to a mutual feedback loop at the level of gene regulation which results in co-regulation of T cell survival and antigen responsiveness (43). This allows CD8⁺ T cells to adapt to the self-specificity of their unique TCRs so that they receive sufficient survival signals without losing self-tolerance. Figure 6B illustrates how the combined TCR and IL-7 survival signal would be expected to change as a function of the strength of interaction between TCR and spMHC in the presence and absence of the coreceptor tuning effect. In the absence of coreceptor tuning, cells with higher self reactivity would receive greater survival stimuli, making them potentially autoreactive. Coreceptor tuning may also allow T cells with a weak responsiveness to spMHC to better receive other pro-survival signals from IL-7.

Studying the circuitry of gene regulatory programs may help bring important new insights into the contribution of IL-7R signaling to autoimmunity and cancer. Exposure of CD8⁺ T cells to low levels of IL-7 *in vitro* for a few hours can pre-dispose them to an enhanced syngeneic mixed lymphocyte reaction with syngeneic DCs (43). This phenomenon suggests that autoimmune phenotypes may involve deregulation of coreceptor tuning machinery. It has also been suggested that elevated responses to pro-survival cytokines may contribute to the ability to avoid death by lymphoblastic leukemia cells (45). The altered responses of these leukemic cells likely reflect critical changes in gene expression. Predicting potential therapeutic targets in the IL-7R signaling pathway will therefore require a comprehensive understanding of how normal control is achieved, and the transcriptional processes contributing to its pathological deregulation.

Multimeric signaling complexes and combinatorial complexity

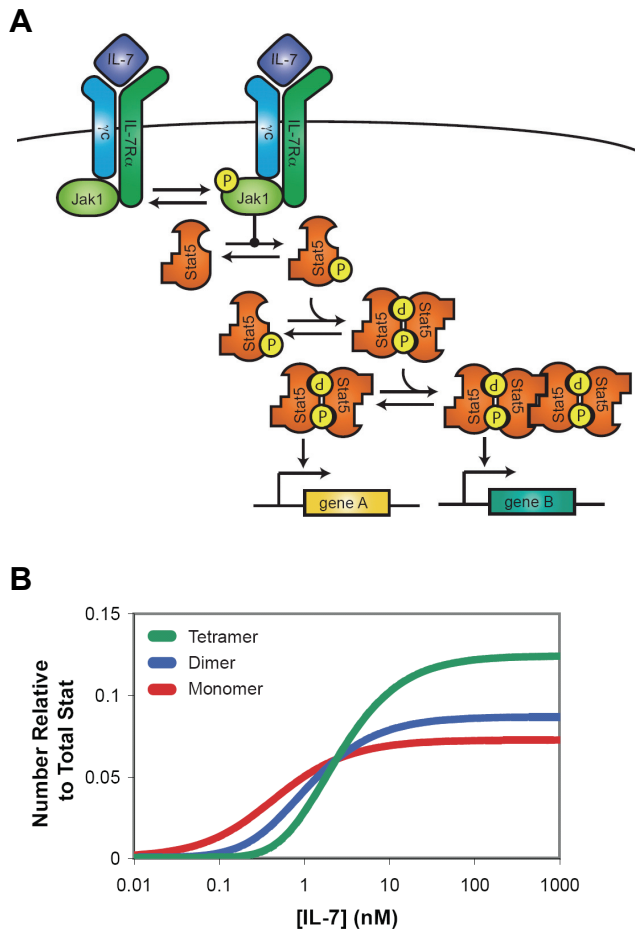The Stat proteins are transcription factors involved in γc

Figure 7. Influence of component multimerization on signaling. (A) IL-7 induced formation of phosphorylated Stat5 dimers and tetratmers, each with unique influences on gene expression programs. (B) Potential Stat5 phosphorylation response showing relative steady state levels of pStat5 monomers, dimers and tetramers with varying IL-7 stimulation dose assuming equal rates of dimerization and tetramerization and first order Stat dephosphorylation. There exist IL-7 concentrations at which dimer abundance is either greater or lesser than tetramer abundance due to a sharper dose response with higher multimerization states.

cytokine signaling that are expressed constitutively at high levels in the cytosol of resting cells which facilitates their ability to induce a rapid response on phosphorylation by Jak kinases (23). Tyrosine phosphorylation of Stats leads to their dimerization through their SH2 domain interactions. Both homodimers (all Stats except Stat2) and heterodimers (Stat1 and Stat2, Stat1 and Stat3, and Stat5a and Stat5b) are formed (23, 32). Furthermore, tetramerization of Stats has been shown to occur in the case of Stat3, Stat4 and Stat5. Such higher order complex formation is mediated by the N-terminal domains of Stat proteins. The Stat dimers and corresponding tetramers could have differences in their respective DNA binding specificities (46).

The presence of several Stat protein multimerization states (monomer, dimer and tetramer) simultaneously present

in the cell, each with different cellular function, complicates the ability to directly relate Stat phosphorylation state to activity and phenotypic outputs. Multimerization kinetics can also cause higher order oligomerization states to be turned on over narrower ranges of input signal (Figure 7). IL-7 has been shown to promote survival versus proliferation at different concentrations (0.1 and 1 ng/ml respectively) in recent thymic emigrants, but the effect is not correlated with total Stat5 phosphorylation (47). One potential explanation could be that Stat dimers and tetramers could preferentially induce cell survival or cell cycle entry respectively. As shown in Figure 7B one possibility is that Stat5 dimers may be more abundant than tetramers upon weak IL-7 signals, with the opposite effect being seen for high IL-7 signals.

Due to cooperative effects, Stat tetramers may selectively increase the activity of certain promoters which have lower affinity binding sites for Stats. Indeed, mutational analysis of Stat5a-binding DNA oligonucleotides has demonstrated that it is possible to introduce specific mutations that virtually abolish Stat5a dimer binding without affecting binding as tetramers. Increased promoter occupancy may change the threshold for transcriptional activity while widening the gene transcription spectrum (46). Some responses to cytokine signaling may be explained by the presence of conditions that favor the generation of dimers over tetramers or *vice versa*. In fact, the presence of high levels of Stat5 tetramers has been consistently seen in some leukemias (47). Furthermore, combinatorial complexity in the dimerization of Stats can lead to additional diversity in the nature of signals transduced by IL-7. Stat5a and Stat5b are two closely related Stats with subtle differences in their DNA binding specificity. These differences are likely to be reflected in the DNA-binding specificities of Stat5a/b homodimers and heterodimers and also in their higher order complexes. Combinatorial complexity in these interactions can also arise from the heterodimerization of other Stats (Stat1/Stat3 or Stat1/Stat2).

Multicellular ligand competition

Lymphocyte homeostasis refers to the maintenance of the numbers and diversity of lymphocytes through an organism's lifetime. The homeostasis of naïve T cells is maintained by the competition for limiting amounts of pro-survival cytokines such as IL-7 and regular contact with spMHC (48). Despite stiff competition for pro-survival ligands among T cells, the homeostasis of T cell numbers as well as diversity is maintained (10). This can be better explained by studying how features of the IL-7R signaling network affect the behavior of the entire cell population.

T cells have evolved strategies to maximize the efficiency of utilization of limiting amounts of IL-7 to maintain homeostasis. IL-7Rα downregulation, which is induced by IL-7R signaling, has been proposed to serve as a mechanism to maximize population size among cells competing for limiting IL-7 by allowing the maximum possible number of cells to receive pro-survival signals (11). In this 'altruistic' model of IL-7R signaling, cells signaled by IL-7 transiently
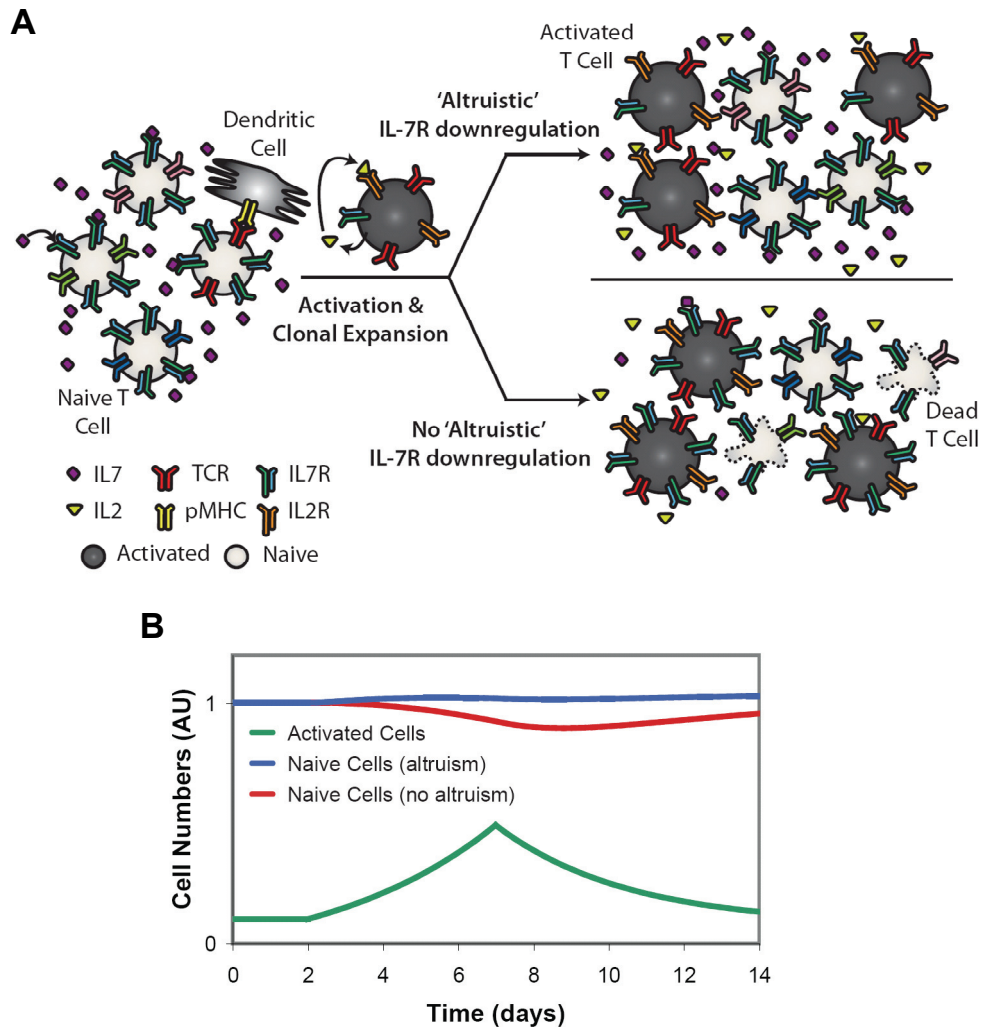
Figure 8. Influence of multicellular ligand competition effects on signaling. (A) Competition between naïve and activated cells in the presence or absence of 'altruistic' IL-7Rα downregulation by activated cells. According to this model, downregulation of IL-7Rα prevents depletion of available IL-7 by the expanding population of activated cells, which is required for naïve T cell survival. (B) An example of how the abundance of naïve and activated T cells might be expected to change once activated T cells undergo rapid IL-7 independent expansion (days 2-7) and either (i) downregulate their receptors ('altruism') or (ii) do not ('no altruism') according to the model in (A). The naïve T cell population sizes decrease in the absence of altruism due to IL-7 ligand consumption.

downregulate surface expression of IL-7Rα and thus become unresponsive to the continued presence of IL-7. The remaining IL-7 in the extracellular milieu can then be used to signal as of yet unsignaled cells. Cells are hypothesized to indefinitely cycle between signaled and unsignaled states with low and high receptor expression respectively (3). It is unknown if such cycling occurs *in vivo* and if it does occur, what the time scales of the cycling in receptor levels are. A further point of contention that remains unexplored is whether 'altruistic' behavior must be realized by two populations of 'signaled' and 'unsignaled' cells *in vivo*, driven for example by their respective arrival at the IL-7 production sites in the lymph node, or whether the entire population is maintained at constant receptor levels in equilibrium with extracellular IL-7. Furthermore, it is possible that the

comparatively faster processes of IL-7Rα internalization are sufficient to regulate signal strength and depletion.

This 'altruistic' behavior has been extended to explain the need for IL-7Rα downregulation upon T cell activation (Figure 8) (11). Activated T cells undergo rapid expansion and their numbers can occasionally reach almost half of the total number of T cells during some acute infections (49). On activation, T cells gain responsiveness to other cytokines such as IL-2 and downregulate IL-7Rα and IL-7 consumption, presumably in order to preserve the naïve repertoire that is critically dependent on IL-7. Figure 8B shows the possible decrease in total naïve T cell population numbers that might be expected in the absence of IL-7Rα downregulation in the activated pool as compared to what would be seen for an 'altruistic' activated pool. A similar interaction is believed to

exist between double negative (DN) and double positive (DP) thymocytes. The DN population of lymphocyte precursors in the thymus is critically dependent on IL-7 and gives rise to DP cells that constitute a large fraction of the cells in the thymus. As forced expression of IL-7Rα on DP thymocytes results in reduced numbers in the thymus, it has been suggested that the physiological downregulation of IL-7Rα on DP cells results in sparing of the limiting IL-7 resource for the DN population (50).

IL-7 has been tested as a therapy for lymphopenic disorders (51). A population level model of the IL-7R signaling network can be used to estimate optimal IL-7 therapeutic dosing regimens that minimize simultaneous activation of negative feedback loops. This will require careful determination of the effects of both receptor downregulation at increasing doses in combination with consideration of the effects of multi-cellular competition.

Conclusions

We have discussed several examples of pathological perturbations in the IL-7R signaling network. However, the involvement of IL-7R signaling in many diseases is not completely understood. For instance, a likely causal single nucleotide polymorphism (rs6897932) in the IL-7Rα gene, was recently identified in a population of multiple sclerosis (MS) patients (52). It results in aberrant expression of a soluble isoform of the IL-7Rα by putatively disrupting an exonic splicing silencer. Simultaneously, the level of the normal membrane-bound IL-7Rα is reduced. This may have an impact on the pathogenesis of MS by altering the dynamics of the IL-7 or TSLP signaling network in multiple cell types. Notably, TSLP-activated DCs are involved in CD4^{+} T cell homeostasis (53) and T regulatory cell development in the thymus (54). In another recent development, a low level of IL-7Rα was shown to be an excellent marker for human T regulatory cells and Foxp3 expression (55). This further suggests that there may be hitherto unrecognized roles for the IL-7R signaling network in human diseases.

The IL-7R signaling network provides a good example of the utility of a system perspective in examining how multiple levels and mechanisms of regulation interact to produce a dynamic cellular response. A view of the pathway that is focused on single components provides an incomplete picture of the regulatory network because IL-7R simultaneously signals through multiple shared pathways and IL-7R signaling is modulated through multiple concurrent mechanisms. We can reliably model the complex biology of IL-7R signaling only by integrating what is known about all the factors involved in signal propagation, repression and modulation. A comprehensive quantitative evaluation of the IL-7R signal network will extend our understanding of complex relationships involved in lymphocyte development and homeostasis. In addition, a detailed network analysis of IL-7R signaling may shed light on other cytokine signaling networks as well because it shares many components with

other cytokine signaling networks. New technologies that allow quantitative network level analysis of signaling will improve our understanding of the immunobiology of IL-7R signaling.

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