The Linker for Activation of T Cells (LAT) Signaling Hub: From Signaling Complexes to Microclusters*

Published, JBC Papers in Press, September 9, 2015, DOI 10.1074/jbc.R115.665869

Lakshmi Balagopalan†, Robert L. Kortum‡, Nathan P. Coussens§, Valarie A. Barr*, and Lawrence E. Samelson**

From the †Laboratory of Cellular and Molecular Biology, Center for Cancer Research, NCI, National Institutes of Health, Bethesda, Maryland 20892-4256, the ‡Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814, and the **Division of Pre-Clinical Innovation, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, Maryland 20850

Since the cloning of the critical adapter, LAT (linker for activation of T cells), more than 15 years ago, a combination of multiple scientific approaches and techniques continues to provide valuable insights into the formation, composition, regulation, dynamics, and function of LAT-based signaling complexes. In this review, we will summarize current views on the assembly of signaling complexes nucleated by LAT. LAT forms numerous interactions with other signaling molecules, leading to cooperativity in the system. Furthermore, oligomerization of LAT by adapter complexes enhances intracellular signaling and is physiologically relevant. These results will be related to data from super-resolution microscopy studies that have revealed the smallest LAT-based signaling units and nanostructure.

An Introduction to LAT-based Signaling

T cells have a central role in adaptive immunity, and their activation involves many signaling processes that are spatially and temporally coordinated. T cells differentiate in the thymus, circulate in the blood and lymphatics, and reside in mature lymphatic organs (lymph nodes and spleen). T cell activation requires physical contact between an antigen-presenting cell bearing antigen and a T cell, whose clonally defined antigen receptor (TCR) recognizes and binds its cognate peptide antigen, which is bound to a molecule encoded by the major histocompatibility complex (pMHC). This initial binding event must then be translated into a productive signal that involves the recruitment and activation of specific molecules within the lymphocyte to generate a successful immune response.

Genetic and biochemical studies have revealed the numerous molecules in the signaling cascades involved in immune function. Activation of the TCR results in engagement of Src family kinases, Lck and Fyn, and recruitment of the Syk family kinase, ZAP-70, to the TCR where, in a process not fully understood, these tyrosine kinases become activated. ZAP-70 then phosphorylates the membrane-bound adapter protein, LAT (linker for activation of T cells) (1). Phosphorylated LAT associates with critical proteins including enzymes and adapters that regulate most TCR-dependent responses. This association with multiple signaling proteins allows LAT to serve as a point of signal diversification and amplification downstream of the TCR. The essential nature of LAT complexes to TCR signaling was revealed by studies in Jurkat T cell lines lacking expression of LAT that are severely defective in several TCR-mediated signaling events (2, 3). In animal studies where either LAT is deleted (4, 5) or knock-in mutations blocking LAT phosphorylation are introduced, thymocyte development is completely blocked (6, 7). Furthermore, deletion of LAT in mature T cells severely compromises T cell signaling (8, 9).

Microscopy Reveals Signaling Microclusters

Microscopy has been vital in bridging the gap between the study of large immune structures such as tissues and cells, and the small signaling complexes identified by biochemistry. Light microscopy has revealed a specialized structure called the immune synapse (IS) formed between T cells and antigen-presenting cells upon successful TCR triggering. The complex structure of the mature IS and its function are reviewed elsewhere (10). Prior to IS formation and within the mature IS, signaling occurs within microclusters, structures of roughly 200–500 nm that are enriched in TCRs, costimulatory molecules, and multiple signaling molecules, including upstream kinases, adapters including LAT, and downstream effectors (11, 12). The enrichment of these signaling molecules in microclusters creates a localized environment in which TCR signaling events can be rapidly propagated to downstream effectors. The functional properties that result from molecular clustering of LAT-containing complexes is a significant question, but one that has been difficult to resolve due to both the essential nature of LAT to TCR signaling (see above) and the technical challenges of distinguishing between the role of individual LAT complexes versus larger LAT clusters.

The spatial structure of the immune system is thus highly organized at many size scales: from the organ level, cells, and macromolecular structures between cells, to microclusters to small protein complexes (Fig. 1). In this review, we attempt to understand the activation of T cells over multiple size scales by focusing on LAT, a protein that functions as a signaling hub in T cell activation. We will first focus on LAT-nucleated protein interactions, and then summarize current views on cooperativity and adapter-mediated oligomerization of LAT, and finally we will review the super-resolution microscopy studies that

---

* This work was supported by the Intramural Research Program of the National Institutes of Health, NCI, Center for Cancer Research (CCR). This is the fourth article in the Thematic Minireview series “Protein Interactions, Structures, and Networks.” The authors declare that they have no conflicts of interest with the contents of this article.
† To whom correspondence should be addressed. E-mail: samelsonl@helix.nih.gov.
‡ The abbreviations used are: TCR, T cell receptor; LAT, linker for activation of T cells; IS, immune synapse; GEF, guanine nucleotide exchange factor; Sos, Son of Sevenless; IDR, intrinsically disordered region; ITC, isothermal titration calorimetry; PRR, proline-rich region; WASP, Wiskott-Aldrich syndrome protein; STED, stimulation emission depletion microscopy; PALM, photo activation localization microscopy; PLC, phospholipase C; SH, Src homology; ADAP, adhesion and degranulation-promoting adapter protein; EGFR, EGF receptor.

This is an Open Access article under the CC BY license.
LAT Nucleates Signaling Complexes

LAT has a very short extracellular region, a transmembrane domain, and a long cytoplasmic region containing multiple tyrosines that are rapidly phosphorylated following TCR stimulation (13). These Tyr(P) motifs serve as binding sites for SH2 domain-containing proteins, including PLC-γ1, Grb2, and Gads (14–16), allowing the nucleation of multiple signaling complexes on LAT, which are essential for downstream signaling. Grb2 and Gads consist of an SH2 domain surrounded by two SH3 domains, which bind to proline-rich sequences in the Ras guanine nucleotide exchange factor (GEF), Son of Sevenless (Sos), and the adapter molecule SLP-76, respectively. SLP-76 further recruits molecules Nck, Vav, and Itk to the LAT complex. Additional molecular interactions are also possible because Grb2 and Gads, via their SH3 domains, and SLP-76, via its Tyr(P) motifs and SH2 domain, can bind other proteins. PLC-γ1 has a catalytic domain as well as peptide and phospholipid interaction domains that are important for Ca2+ influx and protein kinase C activation (17). The modular architecture of protein domains in LAT-associated signaling proteins can be used in a combinatorial fashion to generate flexibility, and provides for the conversion of the same signal input into potentially a large number of different outputs (18).

The juxtamembrane region of LAT contains two cysteine residues, Cys-26 and Cys-29, that are critical for LAT palmitoylation. As a result of palmitoylation, LAT is localized in lipid rafts or membrane microdomains that are resistant to detergents (19). Reflecting the intense debate about the physiological role of lipid rafts (20, 21), the importance of raft localization for LAT function has been controversial. Although early studies using cysteine mutants concluded that LAT localization to lipid rafts was required for function (19), later studies demonstrated that targeting of LAT to the plasma membrane is sufficient for its function, irrespective of its lipid raft localization (22, 23).

In addition to being a positive regulator of T cell signaling, LAT also recruits several negative regulatory proteins, including kinases, phosphatases, and ubiquitin ligases, which ultimately leads to signal termination (17). LAT is also subject to ubiquitylation, and ubiquitin-resistant mutants of LAT display enhanced signaling (24, 25). The above-described protein-protein interactions and post-translational modifications were mapped by candidate coimmunoprecipitation studies to evaluate interactions, one protein at a time. A recent study using mass spectrometry revealed 90 signaling proteins associated with LAT, ZAP-70, and SLP-76, many of which have not been previously known to participate in TCR signaling (26). Thus, the high-resolution power of newly available technologies will lead to a more comprehensive understanding of the LAT signaling hub.

The intracellular region of LAT comprises ~200 residues and has intrinsically disordered characteristics (27). Intrinsically disordered proteins (IDPs) or proteins with intrinsically disordered regions (IDRs) frequently function as hubs in protein-interaction networks due to their physical characteristics (28). The flexibility of IDRs allows for interactions with different partners with high specificity, but low affinity, which enables dynamic regulation of signaling complexes. The inherently unfolded conformation provides accessible sites for post-translational modifications. Because of its unstructured nature, the LAT cytosolic region is likely to have a larger intermolecular interface than a compact, well folded protein, which would improve the accessibility of LAT tyrosines for kinases and bind-
ing partners. Both modular protein domains as well as IDRs contribute to cooperative effects in LAT complex stabilization as described below.

**Cooperativity Stabilizes LAT-based Signaling Complexes**

Cooperative interactions among proteins are characterized by an altered affinity due to multiple binding interactions that influence each other. They can lead to nonlinear feedbacks in biochemical networks (29). A multitude of studies indicate that cooperative interactions among LAT-associated proteins drive the assembly of spatial and temporal specific signaling complexes.

Evidence for cooperativity within LAT-based signaling complexes begins with proteins that interact directly with LAT. Tyrosine phosphorylation of the LAT residues, Tyr-132, Tyr-171, Tyr-191, and Tyr-226, induces interactions with the SH2 domain-containing proteins PLC-γ1, Grb2, and Gads (14–16). A binding preference among these proteins has been observed in co-precipitation or pulldown studies with PLC-γ1 to LAT, likely reflecting the indirect interactions required for the recruitment of individual components (14–16).

Observations of cooperativity within LAT-based signaling complexes have also been made from proteins that interact directly with LAT, including SLP-76 and Sos1. Phosphorylation sites of SLP-76 bind the SH2 domains of the kinase, Itk, and the guanine exchange factor, Vav; however, neither protein will associate with SLP-76 in the absence of the other (32). Furthermore, as detailed below, SLP-76 requires the C-terminal SH2 domain for recruitment into microclusters (33, 34). Interestingly, the C-terminal SH3 domain of Gads and the SH3 domain of PLC-γ1 induce a disorder-order transition in SLP-76, and this may have a cooperative effect on the assembly of the SLP-76 multiprotein complex on LAT (30, 35).

**LAT Oligomerization by Multipoint Binding of Grb2 to LAT and Sos1**

Recently, it has been recognized that multivalency, whereby a molecule can make multiple distinct binding contacts and thus oligomerize signaling complexes, is an important form of cooperativity in cellular signaling (36). In the T cell, Sos1 is recruited to LAT via the adapter Grb2, with the proline-rich region (PRR) of Sos1 interacting with Grb2 via one of its SH3 domains, and the SH2 domain of Grb2 interacting with LAT on any of three phosphorylated Tyr residues (171, 191, and 226) (15). Detailed analysis using mutated forms of LAT showed that at least two of these sites must be phosphorylated for co-immunoprecipitation between LAT and Grb2 (16), suggesting some level of cooperativity in Grb2-LAT complexes, such that individual interactions are destabilized.

Biophysical studies provided interesting insights into the individual and combined interactions between LAT, Grb2, and Sos1. ITC studies using purified Grb2 and phosphorylated LAT peptides showed multipoint binding between LAT and Grb2, such that a fully phosphorylated LAT could simultaneously interact with three Grb2 molecules (37). Furthermore, analysis of singly versus doubly phosphorylated LAT peptides revealed cooperativity upon binding to multiple Grb2 molecules (38), confirming the earlier immunoprecipitation studies. Similarly, ITC between Grb2 and the PRR of Sos1 revealed two distinct Grb2-binding sites on Sos1, such that Grb2 and Sos1 formed a 2:1 complex (37). Moreover, analytical ultracentrifugation studies revealed that Grb2-Sos1-Grb2 complexes could cross-link LAT peptides, suggesting that these complexes could act as a scaffold to promote LAT oligomerization (Fig. 2).

Microscopic analysis of LAT microcluster formation as a surrogate measurement of LAT oligomerization confirmed a role for Grb2 and Sos1 in LAT oligomerization. An ectopically expressed LAT-YFP fusion protein, unable to bind Grb2 (LAT3YF mutant), was not incorporated into microclusters following anti-CD3e stimulation (37). More directly, CD3e-stimulated endogenous LAT microcluster formation was defective following Grb2 depletion in human T cells (39) and in Sos1−/−DP thymocytes (40). In each case, defective TCR-dependent ERK activation, PLC-γ1 phosphorylation, and downstream calcium flux were observed (37, 39, 40). However, it is difficult to attribute these downstream signaling defects directly to a loss of LAT oligomerization, due to the intertwined nature between interactions required for the recruitment of individual components to LAT and those required to promote LAT oligomerization. For example, although the LAT3YF mutation blocks LAT microcluster formation (37), this same mutation blocks the recruitment of both the Grb2-Sos1 and the PLCγ1-Gads/SLP76-Itk complexes to LAT (15, 16). Similarly, depletion of either Grb2 or Sos1 prevents the recruitment of Sos1 RasGEF activity to LAT at the membrane. These overlapping functions make it difficult to determine whether any changes in TCR-dependent development (4–8, 41–43) or signaling (8, 15, 16, 37, 39, 40) observed by LAT deletion, LAT mutation, or Grb2/-Sos1 deletion can be attributed directly to a loss of LAT oligomerization.

**In Vivo Studies Reveal a Role for Sos1-dependent LAT Oligomerization in the Thymus**

To overcome these limitations, we devised a transgenic system that allowed us to independently restore Sos1−/−T cells with either Sos1 RasGEF activity or the ability of Sos1 to nucleo-
ate Grb2-dependent LAT oligomerization (40). A catalytically inactive mutant of Sos1 unable to activate Ras (F929A) was still able to bind Grb2 and promote LAT microcluster formation, allowing the restoration of the Sos1 scaffolding function without its RasGEF activity. In contrast, a fusion construct in which the PRR of Sos1 was replaced with the SH2 domain of Grb2 (Sos-SH2) directly recruited Sos1 RasGEF activity to LAT, but lacked the ability to cross-link LAT molecules and promote its oligomerization.

Assessment of CD3ε-stimulated signaling in isolated thymocytes from Sos1−/− mice expressing these constructs revealed that the two Sos1 "functional domains" signal independently downstream of the TCR. Sos1-dependent Ras/ERK activation required the recruitment of Sos1 RasGEF activity to LAT, but was completely independent of Sos1 scaffolding function. In contrast, TCR-driven LAT phosphorylation, PLCγ-1 phosphorylation, and downstream calcium flux required Sos1-dependent LAT oligomerization, but were independent of Sos1 RasGEF activity.

To determine the functional relevance of Sos1-dependent LAT oligomerization, we further assessed receptor-driven thymocyte development in these mice. At early stages of T cell development in the thymus, a surrogate TCR known as the pre-TCR is employed. Thymocytes must progress and survive through multiple steps, including TCR chain selection, as well as positive and negative selection before achieving full functional status (45). Previous studies had shown a role for Sos1 in both pre-TCR-driven proliferation during β-selection (41) and TCR-dependent negative selection (42) (for a detailed review of Ras signaling during T cell development, see Ref. 46). Assessment of TCR-dependent negative selection in these mice revealed that this process required Sos1 RasGEF activity, but was independent of Sos1-driven LAT oligomerization (40), confirming the Ras dependence of this developmental checkpoint.

In contrast, pre-TCR-driven proliferation required both Sos1 RasGEF activity and scaffolding functions. Restoration of either Sos1 RasGEF activity or its scaffolding function alone failed to restore normal thymocyte proliferation. In contrast, simultaneous restoration of these two signals in trans, accomplished by simultaneous expression of the F929A and Sos-SH2 transgenes in a Sos1−/− background, completely reversed the Sos1−/− phenotype. Both normal pre-TCR-driven proliferation and activation of downstream signaling pathways were observed in these mice, confirming that Sos1 has two independent functions that act in concert downstream of the TCR. Thus, Sos1-mediated oligomerization is functionally relevant in a complex developmental pathway.

**Multipoint Binding of SLP-76 by ADAP**

Combined interactions are also important in recruitment of SLP-76-associated proteins to LAT. SLP-76, which binds indirectly to LAT via Gads and is critical for appropriate T cell responses, modulates signaling complex assembly by direct interactions with enzymes and adapters, including PLC-γ1, Vav, HPK1, Nck, Gads, and ADAP (17). In confocal imaging studies of T cell lines and peripheral blood lymphocytes, these and other proteins were observed in microclusters (47–49). Interestingly, SLP-76 microclusters required both the Gads-binding region and, unexpectedly, a completely different region, the C-terminal SH2 domain of SLP-76 (33). This result indicates that association with LAT is insufficient to incorporate SLP-76 into LAT-containing microclusters. Recently, direct binding of the SLP-76 SH2 domain to three phosphorylated tyrosine residues, Tyr(P)-595, Tyr(P)-651, and Tyr(P)-771, of the adapter ADAP was demonstrated along with the potential for ADAP to oligomerize SLP-76 in vitro (34). These results suggested a mechanism whereby SLP-76 microclusters are stabilized by multipoint binding of the C-terminal SH2 domain to phosphorylated ADAP sites (Fig. 2).

Multicolor confocal imaging studies of Jurkat cell lines supported and extended the multipoint binding model by demonstrating a role for all three ADAP sites in microcluster assembly and stabilization. Single tyrosine to phenylalanine point mutations to any one of the three ADAP sites reduced the total amount of SLP-76 microclusters, co-localization between SLP-76 and ADAP, and the recruitment of both SLP-76 and ADAP into microclusters. Further live cell imaging studies demonstrated a reduction in both microcluster assembly and persistence associated with a loss of the ADAP-binding sites.
MINIREVIEW: LAT-based Signaling Complexes

These results argue that ADAP is critical for the oligomerization of SLP-76. In future studies, it will be important to examine whether multipoint binding of SLP-76 to ADAP also impacts LAT oligomerization. Regardless, it appears that there are multiple mechanisms for oligomerization of TCR activation-induced signaling complexes.

Multivalent LAT and Phase Transitions

As described above, cross-linking of LAT can arise from the formation of a 2:1 complex between Grb2 and Sos1. The 2:1 Grb2-Sos1 complex can bridge two LAT molecules through interactions of the Grb2 SH2 domain with LAT phosphotyrosines on two separate LAT molecules. However, LAT contains nine conserved tyrosines in its cytoplasmic tail, and of these, the distal three are located in YANYX motifs that bind the SH2 domain of Grb2 when phosphorylated (51). Thus, the valence of LAT for Grb2 can vary from 0 to 3 depending on the number of phosphorylated LAT tyrosines.

The distribution of LAT valence states depends on the concentration of the activated kinase ZAP-70 (52), which in turn depends upon TCR activation. Theoretical modeling studies predicted that the valence of LAT for Grb2 is critical in determining the nature and extent of aggregation. Monovalent LAT-Grb2 would block LAT chain formation, whereas bivalent LAT-Grb2 would prevent LAT branching. Thus, LAT aggregation would be significantly reduced in the presence of mono- and bivalent LAT. Such states might be favored by enhanced LAT-Gads-SLP-76 binding as Gads competes for two of the Grb2-binding sites. In contrast, a dramatic rise in LAT oligomerization would occur when the valence for Grb2 switches from 2 to 3, at which point equilibrium theory predicts the formation of a gel-like phase for oligomerized LAT (53, 54).

Phase separation might promote compartmentalization of LAT and LAT-binding partners in transient structures, thus changing their local concentrations. This increased proximity of signaling molecules can greatly facilitate various biochemical processes (55). Local concentration of enzymes in proximity with their substrates and even with each other would promote enzymatic reactions and autoactivation that would not have been possible in solution because of the intrinsic low affinity of the interactions. Increased proximity between molecules in different complexes might enhance signal propagation between adjacent complexes. Moreover, LAT molecules within these compartments might be better protected from tyrosine phosphatases and thereby increase their signaling output.

This sort of phase separation has been shown to occur in other signaling systems as well. For example, in the case of the actin regulatory protein, Wiskott-Aldrich syndrome protein (WASP), a phase transition resulted in a sharp increase in activity toward an actin nucleation factor Arp2/3 complex. In this system, the phase transition was regulated by the degree of phosphorylation of nephrin, a known WASP interactor (56). Thus, in both LAT and WASP systems, kinase activity potentially regulates the extent of oligomerization that then leads to phase transition with functional consequences.

Super-resolution Studies Reveal LAT Nanoclusters

The size of TCR activation-induced signaling complexes is a long-standing question that has been addressed using a variety of imaging techniques. LAT-containing signaling microclusters appear to be 200–500 nm in diameter when visualized on a stimulatory surface by diffraction-limited light microscopy (Fig. 3 A) (11, 12). LAT clusters are very dynamic, and studies describing live cell imaging of LAT as well as the debate about the cellular pool of LAT contributing to T cell activation are reviewed elsewhere (17, 57).

Since LAT microclusters were first visualized, most signaling molecules recruited to LAT have been localized to these structures, thus leading to the realization that microclusters are the primary functional signaling unit. Microclusters have been extensively studied in a wide range of model systems and in ex vivo T cells (58, 59). However, the size and molecular composition of individual signaling complexes within the microcluster are well below the limit of conventional light microscopy, limiting our ability to understand the complexity of signaling events that occur within these structures. Recently, new developments in optical methods have enabled significant improvements in spatial resolution and have allowed visualization of T cell signaling at the nanometer scale.

When viewed using stimulation emission depletion microscopy (STED), a form of super-resolution microscopy reviewed in Ref. 60, most LAT microclusters are clearly composed of smaller clusters in the range of 50–70 nm, near the limit of STED detection (Fig. 3 B). Recently, many researchers have...
turned to single molecule localization microscopy (photo activation localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM), reviewed in Ref. 61) to visualize individual LAT molecules at even higher resolution, and thus determine the size and organization of LAT clusters (Fig. 3C). These studies revealed that LAT is clustered in both unactivated and activated cells and that the extent of clustering increased after TCR activation (62, 63).

Two-color PALM has been used to image LAT pairwise with other signaling proteins. Studies assessing pairwise interaction between LAT and TCRγ have shown conflicting results. In one study, LAT and TCRγ nanoclusters mixed in unstimulated cells. Upon stimulation, LAT nanoclusters only partially overlapped with nanoclusters containing activated TCRγ and ZAP-70, thus forming “hot spots” for LAT phosphorylation (63). These conclusions contrast with another study that emphasized the role of larger “protein islands” of TCRγ and LAT that concatenate, but do not mix upon T cell stimulation (62). In contrast, LAT clusters recruited Grb2 regardless of size, indicating that even small nanoclusters contain phosphorylated LAT. Interestingly, LAT and SLP-76 were reported to form nanostructures with LAT tending to be in the center and LAT. Interestingly, LAT and SLP-76 were reported to form nanostructures with LAT tending to be in the center and SLP-76 distributed on the outside (63) (Fig. 1). How small-scale nanostructures with LAT tending to be in the center and LAT. Interestingly, LAT and SLP-76 were reported to form nanostructures with LAT tending to be in the center and SLP-76 distributed on the outside (63) (Fig. 1). How small-scale organization of LAT structures influences T cell activation is a key unanswered question.

How Do Insights from LAT-based Complexes Apply to Other Systems?

Technical advances in the past few years have transformed our view of LAT complexes and clusters. Super-resolution imaging revealed that the classic LAT microclusters visualized by diffraction-limited microscopy more than a decade ago are in fact made up of LAT nanoclusters that exist in a wide range of cluster sizes in a continuous distribution. In vitro approaches, using purified proteins and biophysical techniques, have uncovered LAT oligomers that have in vivo function. Although all LAT clusters, irrespective of size, get phosphorylated, as evidenced by Grb2 recruitment (as shown by PALM), whether the smallest clusters can lead to productive T cell signaling or whether larger LAT aggregates are required for certain TCR-driven events remains an important question.

Higher-order oligomers have been observed in several signaling cascades (55) and allow for signal amplification, impart threshold response and reduction of biological noise, and render temporal and spatial control of signaling. Several adapter proteins and growth factor receptors have multiple Grb2-binding sites, so Grb2-mediated oligomerization of such proteins themselves might play a role in these signaling systems. In that context, in contrast to the widely held view that dimeric EGF receptor (EGFR) is the predominant signaling unit, some studies have implicated higher-order EGFR oligomers as the dominant species associated with the ligand-activated EGFR tyrosine kinase activation (50, 64, 65). Recent evidence that higher-order oligomers of EGFR bind Grb2 with high efficiency points to EGFR clustering and adapter binding in an oligomeric complex (44).

Key questions for future studies will involve understanding how complex cooperative interactions and oligomer formation control signal outcomes. Tractable in vitro systems can be used to generate binding parameters that can be used to build models describing the behavior of LAT-containing complexes. For example, phase transitions might be a general feature of multivalent signaling systems that impart nonlinearity in signaling pathways. Better definitions of binding parameters could also inform the design of inhibitors of signaling complex formation. Finally, single particle cryo-EM or crystal structure data could give us transformational insights into the structural basis of clustering and cell signaling.

The LAT signaling hub can be seen as a system of heterogeneous molecular organization and dynamic protein assembly, where gaining spatial and temporal information on specific proteins at the nanoscale could yield fundamentally new insights into signaling. These insights might extend to other clinically relevant signaling systems, such as those activated by growth factors, cytokines, or cell-cell contact. For T cells in particular, these insights might have impact on the control of T cells in treatment of autoimmune diseases or graft rejection and the clinical use of T cells in immunotherapy directed at cancer or chronic infection.

Acknowledgments—We acknowledge and appreciate the work of several former members of the laboratory who contributed to the work reviewed in this manuscript. Many important contributions could not be incorporated due to space limitations. We thank Dr. Chang Yi for helpful discussions and Dr. Connie Sommers for critical reading of the manuscript.

References

MINIREVIEW: LAT-based Signaling Complexes


