Membrane compartmentalization during T cell receptor signalling and immunological synapse formation

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COMPARTIMENTALIZACIÓN DE LA MEMBRANA CELULAR DURANTE LA SEÑALIZACIÓN POR EL TCR Y LA FORMACIÓN DE LA SINAPSIS INMUNOLÓGICA

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RESUMEN

La compartimentalización de la membrana celular resulta de interacciones entre proteínas y componentes del citoesqueleto, así como microdominios lipídicos enriquecidos para colesterol conocidos como balsas lipídicas. Estas balsas lipídicas contienen moléculas de señalización y esto les ha hecho candidatos para un papel fundamental en el inicio y el mantenimiento de la señalización celular. No obstante, distintas observaciones han servido para poner en cuestión este papel. Por este motivo, y utilizando el linfocito T como modelo experimental, presentamos aquí avances recientes en nuestro conocimiento de la compartimentalización de la membrana celular durante la transducción de señales. Los linfocitos T ofrecen ventajas únicas para el estudio de este problema dado el conocimiento de las cascadas de señalización utilizadas por el TCR, de la cinética de interacción de este receptor con su ligando, así como de la morfología asociada a la activación de los linfocitos T y que conlleva la formación de una sinapsis inmunológica. En este contexto, hemos examinado las interacciones entre receptores de membrana, microagregados proteicos, y el citoesqueleto que conjuntamente median la formación de signalosomes en microdominios permissivos para la señalización celular.

PALABRAS CLAVE: Linfocitos T/ Receptor para antígeno de los linfocitos T/ Balsas lipídicas/ Citoesqueleto/ Sinapsis Inmunológica/ Transducción de señales.

ABSTRACT

The interaction between transmembrane proteins, lipids, and cytoskeletal components provides a framework for the compartmentalization of the cell surface. Intense research has focused on lipid rafts, the cholesterol-enriched membrane microdomains containing many signalling molecules. However, recent advances in cellular and molecular imaging have challenged prevailing models on the role of these membrane microdomains in signal transduction and their biological significance in cell physiology. Using the T lymphocyte as an example, we review here some of the current developments in our understanding of compartmentalization of signalling. T cells are useful to study this issue given the confluence of knowledge about the morphology associated with early signalling, about the kinetics of antigen receptor engagement, and about the resulting events leading to activation of these cells. Specifically, activation of the T cell upon T cell receptor (TCR) engagement with specific peptide: major histocompatibility complex (MHC) molecule complexes on the surface of antigen-presenting cells (APC) results in a coordinated redistribution of some cell surface proteins into a morphological structure known as the immunological synapse (IS) within a timeline encompassing antigen receptor signalling. In the context of these events, we examine the potential interactions between cell surface receptors, protein-protein microclusters, and cytoskeletal networks that support the formation of TCR-dependent signalling units or signalosomes in signalling permissive environments.

KEY WORDS: T lymphocyte/ T lymphocyte antigen receptor/ Lipid raft/ Cytoskeleton/ Immunological synapse/ Signal transduction.
INTRODUCTION

The spatio-temporal organization of signalling molecules involves their regulated distribution within cellular organelles and compartments\(^{(1)}\). This compartmentalization plays an important role in the initiation, maintenance and termination of signalling within eukaryotic cells. Among the most studied signalling cascades are those occurring inside T cells in response to antigen receptor engagement. T cells are the main mediators of the adaptive immune response leading to specific immunity against particular pathogens. Productive T cell interaction with APCs leading to initiation of signalling requires the coordinated recruitment of signalling molecules (kinases, phosphatases, adapters) within signalling-permissive compartments. Such a productive T cell-APC interaction correlates morphologically with coordinated redistribution of some surface molecules towards the T cell-APC interface and the formation of a structure known as the immunological synapse (IS)\(^{(2-4)}\) and references thereafter).

The adaptive immune response requires the uptake, processing and presentation of antigens by the APCs. Some of the resulting peptides from the processing of these antigens bind molecules of the MHC within the APCs and are presented to T cells scan APCs are scanned by T cells until their clonotypic TCR recognizes specific antigenic peptide: MHC molecule complexes. This productive engagement translates into activation of the src family kinase lck, phosphorylation of the tyrosine-based activation motifs in the CD3 subunits of the TCR complex, and recruitment and activation of the syk family kinase ZAP-70 leading to tyrosine phosphorylation of the transmembrane adapter LAT. Once phosphorylated, LAT provides docking sites for the formation of multimolecular signalling complexes or signalosomes that will trigger downstream pathways\(^{(5, 6)}\). Within this framework, it is apparent that TCR-dependent signalling requires coordinated recruitment and assembly of these signalosomes. Although specific modules that mediate protein-protein interactions are well identified, the molecular basis ensuring the functionality of these interactions remains largely unknown. We need to explain how signalling molecules move in a polarized fashion to the site of receptor engagement, how a signalling permissive environment is established as to include certain molecules and exclude others, and how signalosomes are stabilized so that sustained signalling can occur.

Research on signalosome assembly has largely focused on the movement of proteins into and out of lipid rafts\(^{(7-9)}\). These are membrane microdomains defined on the basis of detergent resistance and their enrichment in sphingolipids and cholesterol. However, recent data suggest that signalosome assembly may occur, at least in the initial stages, independently of this specialized lipid microenvironment, and involve anchoring to adaptor/scaffolding proteins that then facilitate the formation of signalosomes. In this review, we will focus on how T cells coordinate the interaction of signalling molecules between subcellular compartments during IS formation. We will examine some of the evidence supporting the importance of the various subcellular compartments in TCR signalosome assembly, and some of the candidate molecules that may coordinate this assembly process.

COMPARTMENTALIZATION OF THE T CELL SURFACE DURING SIGNAL TRANSDUCTION

A priori, there are three broad interactions that can mediate compartmentalization of the cell surface during signal transduction\(^{(10, 11)}\). These involve protein-protein interactions, transmembrane protein interactions with lipids, and transmembrane protein interactions with cytoskeletal elements, all characterized by different approaches (Fig. 1). Interactions of signalling molecules within lipid rafts have largely relied on the ability to detect these proteins in detergent insoluble cell fractions generated upon sucrose gradient centrifugation. In contrast, protein-protein and cytoskeletal interactions have been characterized by co-immunoprecipitations and the effect of cytoskeleton disrupting reagents. More recently, new imaging technologies have allowed for microscopical analysis of these three types of interactions in real time. While conventional wide-field microscopy or confocal microscopy often do not provide the resolution or timescale imaging required to properly study these molecular compartments in action, FRET imaging, single particle or single molecule fluorescence tracking and atomic force microscopy may offer real time visualization of signalosome assembly\(^{(12-16)}\).

Lipid rafts include a variety of signalling molecules as well as components of the cytoskeleton, making them an ideal candidate platform on which to base the formation of signalosomes and the initiation of signalling. Nevertheless, one needs to remember that much of the biochemical data about lipid rafts comes from their detergent insolubility properties and this may be in itself a limitation in the assessment of their biological relevance. Many TCR signalosome components have been localized to lipid rafts, such as lck, fyn, LAT, ZAP70 and some of the subunits of the TCR complex\(^{(17-20)}\). The importance of the partitioning of signalling molecules in rafts is supported by the observation that their biochemical disruption, most often by removing cholesterol, leads to deficiencies in T cell signalling\(^{(17, 19-22)}\). However, cholesterol depletion has important secondary effects on cell function. It can lead to solubilization of the
plasma membrane and extraction of signalling molecules, like LAT and lck\(^{21}\). It can also alter cellular organelles, thereby effecting protein production and trafficking\(^{23-25}\). In fact, when a milder technique to disrupt lipid rafts employing cholesterol oxidase has been used, conflictive results regarding the role of rafts on signalling have been reported\(^{18, 21}\).

Given the limitations of these biochemical techniques, much of the recent advancements in the study of subcellular microdomains have employed imaging technologies. Electron microscopy provides one approach to directly visualize the lipid raft microdomains employing gold particles coupled to antibodies against raft-specific markers and osmium staining, which stains lipid environments rich in unsaturated fatty acids. Using these techniques, several groups have reported that raft-associated proteins, like LAT or H-Ras, exist in distinct cholesterol sensitive membrane microdomains of an approximate size of 50 nm\(^{26, 27}\). However, commonly used raft markers, like GM1, Thy-1 and GPI-anchored molecules, often do not co-localize within the same membrane microclusters\(^{26-29}\), suggesting the heterogeneity of lipid rafts as membrane microdomains.

The heterogeneity of lipid rafts becomes more apparent during some biological processes. For example, migration of lymphocytes results in the segregation of molecules into the leading edge and the uropod. At the leading edge there is accumulation of GM3-containing lipid rafts with talin and Rho GTPases while at the uropod there is accumulation of GM1-containing rafts with CD44 and CD43\(^{30}\). There is evidence suggesting that variations in lipidic composition can play a role in raft heterogeneity. In T cell lines, LFA-1 localizes to lipid rafts in a cholesterol dependent manner while in primary T cells, LFA-1 localization in lipids rafts is less dependent on cholesterol\(^{31}\). T cell signalling molecules may partition within different varieties of lipid rafts as well. For example, lck partitions within lipid rafts in a cholesterol-

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**Figure 1. A Model for Hierarchical Microdomain Interactions in Immunological Synapse Formation.** Formation of an immunological synapse is dependent on the interplay between different molecular compartments in the T cell. Upon receptor engagement and as signalling is initiated, protein-protein microclusters form and are subsequently immobilized by scaffold proteins linking these microclusters to the peripheral cytoskeleton. As synapse formation proceeds, microdomain reorganization occurs. The coalescence of these protein-protein microclusters by oligomeric ligand interactions leads to the assembly of larger raft microdomains. Oligomerization-mediated raft assembly promotes further tethering to the membrane skeleton and cytoskeletal remodelling leading to signalosome stability and redistribution of molecules into distinct molecular clusters.
dependent manner while LAT partitions within rafts even after destabilization of rafts by partial cholesterol extraction\(^{(32)}\). The different varieties of lipid rafts may serve to segregate receptors and signalling molecules so that only upon activation is there a regulated assembly of the signalosome.

The study of specific molecules and membrane microdomains has been significantly enhanced by the introduction of single particle or single molecule fluorescence tracking\(^{(33)}\). This technique not only allows for the direct visualization of individual proteins or lipids but also their motion under resting conditions and under conditions of activation or receptor ligation. Studies using DOPE, a non-raft unsaturated lipid, have shown that membrane diffusion does not occur according to the randomness suggested by Brownian motion, but instead undergoes what has been termed «hop diffusion»\(^{(33)}\). Hop diffusion is defined as the transiently confined movement of lipid molecules followed by a hop movement into a new confinement zone. In other words, lipid molecules seem to jump from one compartment in the membrane to the next. These intrinsic membrane compartments are likely defined by the underlying peripheral cytoskeleton. If one uses DMPE, a saturated lipid expected to reside within lipid rafts, then one observes lower diffusion rates\(^{(33)}\).

What happens with the movement of single proteins resident in rafts compared to non-raft proteins? Looking at single particle tracking of GPI-anchored, raft-resident proteins (e.g., CD59, Thy-1), some studies have reported diffusion rates and movements that are similar to non-raft phospholipids\(^{(33)}\). However, other studies have suggested the existence of cholesterol-dependent membrane confinement zones where proteins remain trapped for tens of seconds to minutes at a time\(^{(35, 36)}\). Furthermore, when these raft-resident proteins are manipulated, it was found that their drag or impedance to movement was larger than non-raft proteins, and that this drag force decreased with cholesterol depletion\(^{(35)}\), suggesting that lipid raft domains do indeed exist and that raft-resident proteins seem to partition within these cholesterol rich domains.

In the context of the hop diffusion model of cell surface compartmentalization, it becomes important to identify the structures that define these membrane domains. An obvious candidate for this role is the peripheral cytoskeleton. This network of actin fibers underneath the inner leaflet of the cell membrane may be one way to limit the movement of transmembrane receptors, and therefore restricting the hop diffusion of lipids and proteins. The cytoskeleton is finely regulated by actin polymerization, and as such perturbation of this event will alter the movement of molecules within the membrane. Fujiwara et al showed that treatment with agents that mediate actin depolymerization increase the size of the compartment zones and therefore residency time of molecules within each compartment\(^{(33)}\). Disruption of the peripheral actin cytoskeleton also decreases the rate of hop diffusion and as a result increases the diffusion rate of molecules within the membrane\(^{(36)}\). Alternatively, stabilization of actin increases residency time without the concomitant increase in compartment sizes\(^{(33)}\). Putting this evidence together, it is plausible to propose that the peripheral skeleton «fences» the lateral diffusion of molecules. The actin fence has transmembrane proteins acting as pickets that protrude into the membrane outer leaflet, thereby acting on lipids and proteins that are resident in the outer leaflet; thus, its name of «picket-fence» model of cell membrane\(^{(33, 40)}\). These protein pickets would have to interact with the membrane bilayer and the peripheral actin cytoskeleton, providing another example of interaction between compartments.

The «picket-fence» model fits nicely with evidence from the cell biology of signal transduction. The cytoskeleton plays a vital part in the signalling events leading to cell activation. In T cells, the activation processes are accompanied by the remodeling of cortical actin including actin filament nucleation and elongation. Recruitment of lipid rafts to the focal activating point is dependent on proper interaction between the signalling cascades and the cytoskeleton\(^{(41)}\). In fact, patients lacking the connecting elements linking TCR signalling with the cytoskeleton, have immunodeficiencies due to impaired signalling from the antigen receptor secondary to defects in recruitment of ZAP70 (e.g., vav deficiency)\(^{(42)}\) or because of defects in downstream signalling (e.g., Wiskott-Aldrich syndrome)\(^{(43, 44)}\). The interaction between the cytoskeleton and signalling events is symbiotic in that in order to initiate signalling there appears to be a requirement for an intact cytoskeleton at least under certain conditions\(^{(45)}\) and signals that initiate the remodeling of the actin cytoskeleton stem from the TCR itself\(^{(46)}\).

Of increasing importance are recent studies of protein-protein microclusters formed during T cell activation. These microclusters are the morphological indicators of cellular microdomains in which signalosomes are assembled and signalling originates\(^{(47)}\). These protein-protein microclusters form within seconds of TCR engagement leading to co-localization of many important signalling molecules and adaptors (Ick, ZAP-70 and LAT)\(^{(47, 48)}\). Before receptor engagement, these signalling molecules and adaptors have a degree of mobility within the membrane\(^{(45)}\). Upon engagement, these molecules become phosphorylated and this coincides with a great reduction in mobility as molecular associations occur\(^{(49)}\). When signalling is inhibited, recruitment of these proteins to form a microcluster is inhibited indicating
that phosphorylation is essential to facilitate these protein-protein interactions. Localization in lipid rafts is not in itself a determinant of the mobility of a molecule. It seems that these microclusters are static within the membrane, although the actual components are in constant flux in and out of the clusters.

Scattered evidence suggests the existence of lipid membrane microdomains that are not raft-like. The VSV G protein and CD4 were shown to partition within detergent soluble (i.e., non-raft) membrane microdomains in clusters of 100-150 nm implying the existence of molecular membrane compartments that are proposed to be dependent on a protein-based segregation system. In addition, different Ras isoforms have been found to partition within the membrane and cluster in non-cholesterol or non-raft signalling microdomains. Interestingly the non-raft partitioning of the different Ras isoforms was dependent on molecular mediators, in the case of H-Ras the cytosolic protein-binding lectin, galectin-1, stabilized the association to non-raft microdomains, K-Ras partitions to membrane microdomains that are dependent on farnesylation. It seems clear then that molecular compartments are not solely based on lipid raft partitioning, protein-protein microclusters within the «picket-fence» confinement zones, but may also involve lipid-lipid or lipid-protein interactions.

THE IMMUNOLOGICAL SYNAPSE

Upon productive TCR:peptide:MHC interaction, T cell signalling proceeds in a coordinated fashion with cytoskeletal remodeling, redistribution of cell surface molecules towards the interface with the APC, and formation of an IS. The IS is subdivided into two main regions, the central supramolecular activation cluster (c-SMAC) and the peripheral supramolecular activation cluster (p-SMAC). The c-SMAC contains TCR, coreceptors like CD4 and costimulatory and accessory receptors such as CD2. The p-SMAC or outer ring of the IS is enriched for adhesion molecules such as LFA-1 and cytoskeletal proteins like talin. Finally, there are membrane proteins, such as CD43, that are actively excluded from lipid rafts.

Formation of a mature IS involves TCR signalling. As T cells scan the surface of an APC, they interact with adhesion molecules, which slow down their movement. Upon recognition of a nominal peptide:MHC complex and interaction with ICAM-1, a stop signal is transduced to the T cell. Following this stop signal, calcium signalling occurs leading to recruitment of signalling molecules (lck, ZAP70 and PI3K) to the T cell:APC interface. Sequential activation of lck and ZAP70 leads to phosphorylation of LAT. LAT itself is targeted to lipid rafts by acetylation/palmitoylation. Tyrosine phosphorylation of LAT provides docking sites for the assembly of signalosomes. This assembly process correlates with the formation of protein-protein microclusters independently of interactions with the cytoskeleton and lipid rafts. The microclusters create protein microdomains that concentrate (e.g. PLCγ1) or exclude proteins (e.g. CD45). The microclusters formed with LAT, lck, ZAP70 and the TCR complex seem to be sufficient for the initiation of signalling leading to calcium flux, phosphorylation of downstream proteins and adaptors, and activation and translocation of transcription factors.

During IS formation, there is a shift from early events centered on the TCR/CD3 complex to events centered on transmembrane adaptors such as LAT and involving the assembly of signalosomes. Phosphorylated LAT recruits signalling molecules such as phospholipase C γ-1 (PLCγ-1) and other adaptors Grb2 and Gads. PLCγ-1 cleavesPIP2 into IP3 and DAG leading to calcium influx and activation of PKC, respectively. Grb2 goes on to recruit SOS and activate the Ras/MAP kinase pathways, while Gads recruit SLP-76 which associates with Vav, Nck, WASP and PAK, themselves regulators of actin reorganization and cell polarization.

The geometric distribution of engaged TCRs during IS formation likely plays a role in the sustainability of signalling from the microclusters. Initially, upon TCR engagement, the protein-protein microclusters centered on LAT are found in the periphery of the developing IS. As the IS matures, these signalling microclusters gravitate towards the c-SMAC and their signalling ability is down-regulated. While the microclusters in the c-SMAC have attenuated signalling, a ring of these signalling microclusters around the c-SMAC maintain their ability to signal. Therefore, the developing IS acts as a control mechanism to insure the sustainability of signalling and attenuate any overly active signalling.

Indeed, architectural constraints on TCR localization within the IS (limiting them to microclusters in the periphery of the forming IS) leads to prolonged signalling. It is important to note that despite the static geographic patterns observed in the IS, there is a continuous and dynamic exchange of molecules occurring. This pattern of a static spatial organization while maintaining a dynamic molecular exchange is also in effect within individual signalling microclusters. While it appears that protein-protein networks do not involve lipid rafts, considerable debate remains as to the
function of lipid rafts in early TCR signalling and IS formation. Many members of the early TCR signalling machinery partition within lipid rafts and this selective partitioning may also serve as a basis for the initiation of the assembly of the TCR signalosome upon TCR ligation. The current paradigm of lipid raft biology has incorporated its dynamic status. Under resting conditions, rafts range in size from 25 nm to 150 nm and contain very few proteins. In resting T cells, the Src kinases Lck and Fyn, ZAP-70, LAT and at least some of the components of the TCR are located in rafts. It is believed that upon TCR engagement with MHC-peptide, rafts fuse to form larger domains allowing the Src kinases to phosphorylate the tyrosine-based motifs in the CD3 chains that subsequently recruits ZAP-70, which in turn, upon activation, phosphorylates LAT. The importance of rafts in T cell activation via the TCR is primarily supported by the observation that disruption of lipid rafts inhibits T cell activation.

Lipid rafts accumulate at the IS, specifically in the c-SMAC, progressively recruited from the periphery of the IS. Given the association of lipid raft microdomains with receptor internalization, one can argue that this profile of localization reflects ongoing TCR internalization. There are discrepancies regarding the importance of raft partitioning for the activation of signalling molecules. For example, LAT localization in rafts via palmitoylation was considered essential for its phosphorylation and subsequent T cell activation. However, a recent report suggested that this may not be the case. Thus, although recruitment and partitioning of signalling molecules within lipid rafts may be required for optimal TCR signalosome assembly and signalling, protein-protein microdomains may be able to exist outside of the lipid raft environment and these may be sufficient to sustain activation of signalling molecules.

The interaction between signalling proteins within lipid rafts and the cytoskeleton has been recognized for quite a few years due to the many correlates observed upon natural impairment (e.g., Wiskot-Aldrych syndrome) or after pharmacological intervention on cytoskeletal function. One of the most studied pathways linking the TCR with the cytoskeleton is the WASp/Nck cascade. While none of the WASp/Nck proteins appear to reside in lipid rafts under resting conditions, they are recruited there upon activation. The WASp/Nck cascade links TCR signalling and cytoskeletal reorganization, with Nck playing a conserved adaptor role, not restricted to immune cells, determining cell morphology in response to signalling. Within the TCR signalosome, SLP-76 interacts with LAT, mediating the recruitment of Nck to the TCR complex. Once part of the TCR signalosome, Nck interacts with a variety of proteins including WASp, regulating actin polymerization via Arp2/3.

There is a tendency to equate the IS with the only site for active signalling during T cell activation. However, the study of the ezrin/mixin/moesin (ERM) proteins during T cell activation and its binding partners focused our attention in the antipodal pole or distal pole complex as a compartment with potential relevance during TCR-induced T cell activation. Antipodal localization has been reported for some surface receptors, and for some second messengers. For example, following TCR signalling, some cell surface receptors such as CD43 and CD44, and specific pools of ERM proteins that anchor these receptors to the cytoskeleton re-distribute to the antipodal pole of the T cell. Following TCR ligation, the second messenger PIP3 not only accumulates in the T cell-APC interface but diffuses into the antipodal pole of the T cell. Also, one of the enzymes that breaks down cyclic-AMP, phosphodiesterase 4B2, redistributes from areas proximal to the IS to the antipodal pole as TCR signalling proceeds. Taken together, this evidence indicates that the antipodal pole of the T cell may be an important signalling site during T cell activation, warranting further study of this site.

CANDIDATES TO MEDIATE INTERCOMPARTMENTAL INTERACTIONS

The involvement of three different compartments (protein-protein networks, cytoskeleton, and lipid rafts) involved in the assembly of TCR signalosomes raises the issue of molecular regulation of this process (Fig. 1). It is plausible to suggest that there is a molecular machinery to coordinate the function of these components. This could take the form of a protein or family of proteins that is able to interact with all three compartments, and that modulates TCR signalling. A few potential candidates to fulfill this role in T cells during TCR signalling will be discussed here (Fig. 2).

One of the protein families implicated in mediating interactions between cell surface receptors and the cytoskeleton in various subcellular compartments is that of the ERM proteins. ERM proteins have an N-terminal FERM domain that associates with the membrane and a C-terminal domain that binds actin. The ERM proteins partition to the lipid rafts and a pool of these proteins cluster at the IS during...
TCR ligation\(^{84, 87}\), although other pools of ERM are seen at the edges of the IS or even at the antipodal pole of the T cell, likely in association with receptors excluded from the IS (e.g. CD43)\(^{83, 84, 88, 89}\). While there appears to be no direct interaction between the ERM proteins and the signalling components of the TCR signalosome, the ERM proteins play an important role in membrane compartmentalization during signalling by linking cell surface receptors with the actin cytoskeleton\(^{83, 84, 89, 90}\). Dephosphorylation of ERM leads to a «thawing» that allows for membrane fluidity by uncoupling of membrane microdomains from the actin cytoskeleton\(^{89}\). This dephosphorylation of the ERM proteins is concomitant with the removal of CD43 and CD44 from the forming IS\(^{83, 88}\). The dephosphorylation of the ERM proteins is followed quickly by rephosphorylation on a timescale of 3 minutes\(^{83, 89}\) which is believed to allow for the reattachment of the membrane to the cytoskeleton. As mentioned, a pool of the ERM proteins accumulate in the IS\(^{87, 90}\), but they also localize to the distal pole complex\(^{82, 83, 88}\). The importance of the ERM proteins in IS formation has also been illustrated using a dominant negative form of ezrin, which leads to increased numbers of APC-T cell conjugates\(^{89}\) but perturbed TCR clustering in the c-SMAC\(^{90}\).

Another candidate regulator of signalosome assembly and stabilization is the reggie/flotillin family of proteins. This is a large family of proteins also known as the SPFH (Stomatin/Prohibitin/Flotillin/HflK/C) proteins, located in lipid rafts, and of unknown function. Preliminary evidence supports a role for these molecules in the interplay between protein-protein microclusters, lipid rafts and the cytoskeleton. The reggie/flotillins have been found in association with lck, fyn and LAT\(^ {91, 92}\) suggesting that they can interact with the TCR signalosomes. Furthermore, associations of reggie/flotillins with LAT, lck, Nck, and vimentin, a cytoskeletal protein, increase after stimulation\(^ {78, 91-93}\). In keeping with a scaffolding role for these proteins, the reggie/flotillins...
redistribute during activation of the T cell to sites of TCR engagement and IS formation\(^{91, 92}\). Thus, reggie/flotillins are key components of those microdomains containing signalling molecules. When deletion constructs that interfere with reggie/flotillin oligomerization are introduced into a cell, defects in cytoskeletal rearrangement appear\(^{94}\). This is in line with evidence indicating that members of the SPFH family of proteins can interact with the cytoskeleton, and contribute to signalling along the Ras-ERK pathway\(^{99}\). We have preliminary evidence showing that members of this family are involved in lipid raft-associated signalling during TCR-dependent T cell activation, and may be critical for the effector function of these cells (Kirchhof MG and Madrenas J, unpublished data).

**CONCLUDING REMARKS**

The structural aspects of signal transduction through antigen receptors continue to be a major unresolved issue in Immunobiology. Three main components seem to play distinctive roles in a temporally and spatially regulated manner: protein-protein networks, cytoskeletal components and lipid raft microdomains. The identification of the sequence of events and their spatial visualization with novel imaging technologies should provide fundamental information for an integrated view of TCR-dependent signalling. Likely it will also raise new questions regarding the regulation and stabilization of TCR-dependent signalosomes, while assigning functional roles to still unidentified players in this process.

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