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Testing the organization of the immunological synapse

Editorial overview

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Matthew Krummel is an associate professor of pathology at the University of California at San Francisco. His lab studies T cell dynamics, in particular the mechanisms for motility arrest, synapse formation, and establishment of cell polarity. For many years, his lab has been interested in novel methods for acquiring and analyzing real-time information about molecular and cellular dynamics in these cells.

Upon encounter with antigen-bearing presenting cells, T cells initiate the formation of a unique junction termed the immunological synapse (IS). The morphology of this junction, characterized in EM as a series of discrete contacts into which are interspersed synaptic spaces [1], has multiple similarities to neuronal synapses. The closely apposed membranes permit engagement of T cell receptors (TCR) on T cells with peptide-major histocompatibility complexes (pMHC) as well as distinct contacts for other receptor–ligand pairs such as integrins. The form of this synapse has also been extensively characterized at the level of molecular organization. When TCRs or pMHC complexes are attached to fluorophores, it is found that they first form dynamic and relatively small structures (clusters or 'microclusters') all over the contact area and perhaps predominantly at the outskirts of the synapse [2–4]. These ultimately coalesce [3,4] to a central spot, and this spot corresponds to a structure first characterized by Kupfer and co-workers in fixed couples and termed the central 'supramolecular activating cluster' or cSMAC [5].

The name cSMAC is perhaps misleading since early calcium imaging together with TCR visualization as well as antibody-staining for phosphotyrosine has shown that signaling onset coincides with the appearance of the smaller clusters, before their centralization [3,6]. As the cSMAC is the last spot from which TCRs are likely to be internalized, it is also the point at which TCR signaling is likely to cease [7]. The cSMAC is surrounded by a slightly externalized distribution of CD4 [3] and by a larger annulus of integrins such as LFA-1 in a peripheral region (termed the pSMAC). The multiple zones of proteins in the IS define distinct membrane domains, and the nature of the domains and their function is the overall theme of this issue.

The IS involves events that are at once dynamic (involving the cessation of motility, repolarization, and coalescence of receptor clusters) as well as having relatively stable aspects. In the past year, Vale and co-workers used a model system in which T cells were triggered by immobilized antibodies to demonstrate that TCRs are confined to relatively stable zones that behave as if they are bounded by a diffusion barrier [8]. This suggests that while the pathway from an unstimulated cell surface to a highly ordered cSMAC/pSMAC array is highly dynamic, it is also highly aided by stabilizing forces in the membrane or in the cytoskeleton. As it is studied more, it becomes clear that the dynamic assembly of the IS relies upon a confluence of biophysical processes within the membrane, and cell biological processes in the membrane or within the cytosol. Recent efforts have focused upon all of these. The foremost structure being studied is the lipid bilayer and the proteins arrays that assemble within this unique environment.

What is happening at the submicron level to membrane receptors, membrane patches, and lipids themselves during the synapse establishment process? What are the distinct outcomes of particular submicron organizations? In this current techniques section of *Current Opinion in Immunology* four groups describe emerging approaches to the problem of measuring and probing the order of the synapse. All address related yet distinct aspects of the surface topology, and all describe emerging technologies for asking key questions.

A fundamental component of the synapse is the lipid bilayer into which integral membrane proteins are threaded. Our understanding of this complex environment continues to evolve, and a popular notion of 'rafts' or 'islands' has garnered experimental support. In this model, the lipid bilayer is not homogeneous, but instead collections of proteins are associated with specific types of lipids. Much of the evidence favoring this model initially came from biochemical fractionation methods involving 'floating' detergent-insoluble material on a density gradient. The meaning of many of these studies is now frequently questioned as it is found that nonraft-associated proteins associate with the floating layers and that raft-like behavior in the synapse is not predicted by this assay ([8] and reviewed in [9]). However, the use of dyes or GFP-fusions targeted to specific lipid moieties and the application of FRET-based technology have reinforced certain aspects of this model. EM approaches toward assessing membrane structure have also suggested that the majority of proteins are always confined to 'islands' in the membrane that are bordered by relatively protein-sparse regions [10]. Gaus and Harder have recently pioneered another approach that relies upon indicator dyes. Using the liquid-order sensing dye Laurdan, they demonstrated that liquid-ordered (Lo) lipids are specifically enriched at the IS [11]. In their review, the lessons of this useful dye are discussed and placed into context of previous studies. As an approach, the use of membrane-sensing dyes holds much promise, and it is likely that Laurdan is the first of many such tools.

Another recent development toward assessing the IS is the use of patterned surfaces to create immobile or mobile arrays that either mimic or counteract cSMAC formation. This issue contains two reviews from the two leaders in this field and its application to the synapse. Irvine and colleagues describe the use of photopolymer masks, stamps, and deposition methods toward creating surfaces that can activate T cells. They review the evidence that the annular nature of the immunological synapse may contribute toward the efficiency of activation and define the technologies and techniques that are currently available to deliver spatially well-defined inputs to a T cell through a synapse-like contact. This follows a recent publication [12] in which it was shown that an inverted synapse, in which TCRs are held outside the IS, results in a destabilized and oscillating contact.

DeMond and Groves describe complimentary and related methods to interrogate synapse structure. In the past year, this group pioneered a method of forming bilayers of pMHC and ICAM complexes whose diffusion properties are limited by physical barriers manufactured into the support. In this way, initial clusters of T cell receptors were kept from centralizing. Interestingly, this resulted in a prolongation of phosphotyrosine signaling [13], providing the strongest evidence to date that centralization of TCRs is their endgame for signaling. As a means to progress from this point forward, they also discuss a novel method for spatial and temporal patterning of ligands for the TCR, using caged ligands that can be spatially 'turned on' by illumination with a burst of UV. Finally, an emerging issue of membrane curvature is briefly touched upon. This latter feature of true immunological synapses — that they occur on membranes that are dramatically curved both at the synaptic cleft as well as at their edges — is one that is underappreciated, yet most certainly contributes to both protein and lipid behaviors.

Finally, Treanor and Batista describe the emerging application of single-particle tracking and FRET in the context of synapse membrane biology. They further address this in the context of in silico modeling. Batista's group recently has shown that for B cell synapses, a membrane spreading event assists in prolonging the signaling response [14]. Part of the means of showing this was in silico modeling followed by testing the prediction of the model experimentally. One of the promises of modeling is indeed in assisting to guide experimental studies. Modeling and theoretical descriptions of individual proteins as well as the unique behavior of cohorts can play a significant part in the complete understanding of a process. It is then a bit unfortunate that some modeling has been put to the relatively trivial exercise of picking a small collection of known parameters and simulating their behaviors until they produce the experimentally defined result. Much more powerful has been the modeling that either predicts unrecognized experimental results or, in some cases, defines a key experiment that will differentiate between two models. As an example of the latter, Figge and colleagues have generated predictive models on the basis of work of Groves [14]. By defining two simplified mechanisms that might drive synapse coalescence, they have defined experiments whose results might differentiate between the two broadly divergent mechanisms [15]. While this type of modeling is still ultimately limited by the imagination of the modeler and/or the complexity of biochemistry (are there really only two options?), it is clear, as discussed by Treanor and Batista, that it serves as a crucial fulcrum upon which many experimental methods can balance.

There is no doubt that the coming years will bring further advances in the techniques and methods by which we understand synapse behaviors. From the instrumentation point of view, there continue to be improvements in the sensitivity of detectors that permit detection of single molecules or small cohorts. Spatial photoactivation of ligands, combined with novel lipid-binding dyes, will further help us to differentiate the contribution of membrane composition toward synapse architecture. These may also prove useful for a related approach — imaging of T cell activation *in vivo*. Together with newer materials and submicron patterned surfaces, perhaps those also incorporating complex surface topologies, these methods are likely to promote the ability to probe the IS with ever-finer detail.

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