Liquidity in immune cell signaling

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T lymphocytes of the immune system need to integrate myriad signals to decide on life and death matters in defending the host from pathogens and distinguishing normal and transformed host cells while minimizing immunopathology. In this issue of Science, Su, Ditlev et al (*1*) provide insight into how phase separation of signaling adapters in the 2D liquid of the plasma membrane enables these critical decisions.

Phase separation in live cells has become a useful concept in explaining the formation of a variety of µm scale spherical, liquid-like compartments that are held together by weak, rapidly reversible interactions and undergo exchange of many components with the surrounding bulk phase, while maintaining a distinct composition and sharp boundaries (*2*). Phase separation is also well studied in 2D system such as biological membranes where co-existing liquid disordered (ld) and liquid ordered (lo) phases are important for signaling by many trans-membrane receptor systems (*3*), including the T cell antigen receptor (TCR) signaling process. Su, Ditlev et al. study how a soluble and membrane anchored adapters proteins down-stream of the TCR combine to form a 2D phase separated system that depends fully on protein-protein interactions on top of a ld bilayer.

They started their analysis by looking at the behavior of a critical signaling effector of the TCR –phosphorylated linker of activated T cells (pLAT) and two of its polyvalent binding partners, Grb2 (which has a central SH2 domain that binds pLAT) and Sos (which has polyproline motifs that bind the N- and C-terminal SH3 domains of Grb2) (*1*). These proteins are critical for Ras and phospholipase Cγ activation down-stream of the TCR. They discovered that physiological levels of these proteins formed µm scale pLAT clusters on SLB in vitro. The pLAT clusters generated in the presence of Grb2 and Sos have characteristics of liquid phases including that they fuse with each other while maintaining a roughly round shape and undergo rapid exchange of pLAT between the pLAT cluster and the surrounding percolating phase. Su, Ditlev et al then expanded the system of purified proteins to include upstream kinases and phosphatases, including CD45, which was partly excluded from the pLAT rich phase based on charge repulsion. Finally, the authors overlay an F-actin polymerizing system on the LAT clusters and find that the 2D pLAT phases can interface with the actin nucleating factor N-WASP to reshape the liquid pLAT phase based on the elongated F-actin gel- a solid. In this context the liquid pLAT rich phase facilitates the formation of the solid F-actin gels and then effectively fills it- like any liquid- taking the shape of the container.

The pLAT clusters are reminiscent of TCR “microclusters”, where signaling reactions take place on the cytoplasmic face of the T lymphocyte plasma membrane, which are

highly dynamic and display fusion events to form larger clusters (*4*). Importantly, TCR microcluster formation in response to physiological ligands is dependent upon F-actin remodeling. Its possible that the complex interaction between the pLAT rich phase and F-actin is related to this requirement and feeds back on the extracellular interactions of TCRs with their ligands, peptide-MHC complexes on the surface of antigen presenting cells.

Concepts of 2D phase separation can also be applied to reconstituted cell-adhesion systems. The adhesion of T cells expressing the receptor CD2 to supported bilayer (SLB) presenting its ligand CD58 display characteristics of phase separated system with the precisely apposed membranes contributing the necessary dynamic multi-point attachments (*5*). Furthermore, different size receptor systems that bridge the gap between the T cell and SLB further subdivide the interface into TCR and adhesion molecule rich domains of the immunological synapse (*6*), the natural setting for the reactions studied by Su, Ditlev et al. The actino-myosin based transport system that drives centripetal transport in the immunological synapse may move the TCR/pLAT clusters using the F-actin based container reconstituted by the authors. The large trans-membrane tyrosine phosphatase CD45 is excluded from the T cell microclusters, which depends on its large extracellular domain (*7*). In this study another critical determinate of CD45 exclusion was found to be the cytoplasmic domain that is also inhibited from entering pLAT clusters; likely due to charge repulsion. The data presented here provides a possible rational for the activation of T cells by extensive cross-linking of the TCR with antibodies, where size based exclusion of CD45 by its extracellular domain is less likely, but instead it may be the coalescences of pLat clusters that protects phosphorylated sites.

Su, Ditlev et al focused on phenomena that were largely confined to the 2D SLB surface by tethering key components to the SLB surface. Its not clear if the 2D phases of pLAT described here could also nucleate 3D phase separated structure that could be released into the cytoplasm. Such structure appear to be formed during T cell stimulation by immobilized anti-TCR antibodies, where SLP-76 foci are seen to be released from the TCR clusters and trafficked toward the microtubule organizing center (*8*). Once at the center of the interface these particles appear to dissipate.

Results of Su, Ditlev and colleagues provide insights into otherwise hard to reconcile characteristics of TCR signaling. Recent super-resolution studies suggest that substrate adherent T cells display basal segregation of TCR and LAT into distinct “protein islands” on a ~100-300 nm length scale (*9*). These islands may be more like oil droplets in water than solid islands. The more solid islands and rafts emerging from the solid gel like cytoskeleton. Its possible that the interaction between different dynamic liquid phases in extracellular face, plasma membrane, and cytoplasmic face of the membrane with the solid scaffolding layer of the cytoskeleton generate emergent features that are otherwise impossible to understand (Figure). The segregation of TCR and pLAT protein islands observed by Lillemeier et al may only be explained by interactions between layers. The tools generated by Su, Ditlev et al have the potential to address these emergent properties of the dynamic, layered circuitry of trans-membrane signaling.

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