T cells like a firm molecular handshake

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T cell activation is one of the most extensively studied intercellular recognition processes. Adaptive immune responses are initiated when a T cell interacts with major histocompatibility complex molecules bearing mixtures of different peptides (MHC peptide) on the surface of an antigen-presenting cell through formation of an immunological synapse (1). The presence of just a few functionally defined agonist MHC peptide complexes in the synapse initiates the T cell response (2). Although CD4 or CD8 coreceptors also interact with the MHC molecules, the binding sites are some distance away from the antigen peptide. The only known direct physical contact the peptide has with the T cell is through the T cell antigen receptor (TCR). This interaction is analogous to a handshake where the interacting binding sites are tethered to large bodies. How could TCR tell different peptides from such a handshake? The kinetic-proofreading model suggests that signaling events downstream of the TCR–MHC peptide interaction require time for assembly and reaction such that the half-lives of the TCR–MHC peptide complexes can be used to rank the biological efficacy of different agonist MHC peptide complexes (3). However, numerous exceptions have been observed in which the potencies of MHC peptides to trigger T cell activation do not correlate with the half-lives of their interactions with TCR. A recent study (4) has shown that multiplying the half-life with the heat capacity change between the bound and free states could bring several outliers into line, but the physical basis of this correlation was not clear.

Synaptic Chemistry

A major caveat of all of these efforts is that interaction measurements are made in solution with disembodied TCR and MHC peptide complexes, but the TCR and MHC peptide complexes interact while tethered to cell bodies in a cell–cell synapse (5). How does the architecture of the immunological synapse impact molecular interaction, and can one extrapolate the properties of molecular interaction in a cell–cell synapse from quantities measured in solution? In this issue of PNAS, Qi *et al*. (6) propose that TCR–MHC peptide interactions that go through conformational changes to form a rigid complex have a particular advantage in achieving a long-lived interaction in the interface compared with in solution.

Compared with interactions in solution (3D), the two most obvious differences of interactions across two apposing membranes between receptor–ligand pairs that are tethered to large objects like cells (2D) are that (*i*) the interactions can be subjected to large forces and (*ii*) the receptor- and ligand-binding sites are subjected to different transport constraints (7). The issue of how receptor–ligand interactions in interfaces react to forces has been intensively studied, revealing that receptor–ligand pair-specific properties allow some adhesion systems to resist separation by forces. Bonds that have these special properties are called "catch bonds" because they seem to dissociate more slowly when they are subjected to external forces than when they interact in solution (8). The "hands" grasp more tightly when the bodies are forced apart. It is not known whether TCR–MHC peptide interaction exhibits catch bond behavior or, if so, whether TCR uses different catch bonds to discriminate different MHC peptides. There are different ideas about the specific molecular features that lead to catch bond behavior, but it is likely that this is a property specific to molecules whose function has a mechanical role (9). Catch bonds are implicated in processes such as leukocyte rolling on vessel walls and bacterial adhesion in the urinary tract, situations where small numbers of receptor–ligand interactions can be subjected to large forces due to flow (8, 10). Forces applied to TCR–MHC peptide complexes in the immunological synapse are likely to be much different because, even when TCR–MHC peptide clusters are moved through the synapse, they are in small groups that move at ≈1 μm/min such that smaller forces are distributed to many receptor–ligand pairs (1, 11).

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Several transport constraints imposed by interactions in 2D have been discussed (5). Restricting the receptor–ligand-binding sites in the confinement layer can increase their local concentration and enhance rebinding, thereby making interactions with low 3D binding affinity more effective binders in 2D (12, 13). Receptors anchored to the cell membrane can be carried by the moving cell to ligands anchored on another cell or on a substratum, which is a form of convective transport (14). Although one would not rule out a biologically important role for the above transport mechanisms, Qi *et al*. (6) suggest that restriction of translational and rotational movement may lead to differences between 3D and synaptic 2D interactions for TCR.

Rigid Interactions Last Longer

One way to treat the restricted rotational movement of membrane-tethered molecules is to quantify changes in the degrees of freedom of the receptors and ligands from the free state to the bound state. All of the prior theoretical and experimental work on cell adhesion had considered the "external" degrees of freedom involving translation and rotation of the molecule in space. The prerestriction of these degrees of freedom by membrane tethering generally enhances interactions at a synapse because interactions of membrane-tethered receptors and ligands result in fewer lost degrees of freedom, meaning less entropy loss and greater effective affinity compared with the same receptor–ligand interaction in solution (13). These effects are not sensitive to any of the thermodynamic or kinetic properties of the receptor–ligand interaction that can be measured in solution. Qi *et al*. (6) for the first time consider how receptor–ligand specific "internal" degrees of freedom could impact the kinetics of the receptor–ligand interaction because conformational changes are associated

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with internal degrees of freedom. In other words, they examined how molecule-specific features influence differences between the cell membrane environment and measurements of half-life in solution.

The starting point for the calculations of Qi *et al*. (6) is the model that the signaling interactions downstream of the TCR–MHC peptide interaction require time for assembly and reaction such that the half-life of the TCR–MHC peptide complexes in the synapse will govern the integrated signaling response. Krogsgaard *et al*. (15) plotted the solution half-life of TCR–MHC peptide interactions in solution versus stimulatory potential and found that there were many outliers, suggesting in this framework that solution or 3D half-life is not always a good predictor of synaptic or 2D half-life. They observed, however, that when they simply multiplied the half-life by the change in heat capacity upon binding, a better correlation with T cell stimulation was obtained. This finding suggested that heat capacity change, a thermodynamic property, was acting as a conversion factor for 3D to 2D half-life, but the physical basis of this effect was not clear. Qi *et al*. (6) reasoned that the loss of rotational degrees of freedom in the membrane environment would increase the barrier to formation of the transition state in the 2D system if the internal degrees of freedom that must bind to form the transition state are relatively rigid. Using statistical mechanical calculations with the approximation that the internal elements are deformed to a small extent and so can be treated as springs, they obtained a formula relating the half-lives in solution to that at a cell–cell junction. This theory suggests that the 2D half-life is proportional to the 3D half-life multiplied by the exponential of the product of the heat capacity and another constant reflecting the rigidity of the internal degrees of freedom. This relationship shows that, if the internal degrees of freedom are relatively flexible, the half-life measured in solution is pretty close to that relevant at a cell–cell junction. Conversely, when the internal degrees of freedom are rigid, the corrected half-life in the membrane environment depends upon the specific heat change upon receptor–ligand binding. Application of this theory to the data resulted in excellent fits of 3D half-life $\times \exp[-B\Delta C_p]$, where B is related to rigidity with stimulatory potential.

These results add to the existing arguments for an evolutionary rationale to keep the TCR and MHC peptide molecules relatively short and rigid at ≈7.5 nm each (16). Prior work would predict that the relative inflexibility of the short rigid molecules decreases the entropy loss on binding and also increases the confinement to increase the 2D affinity. This confinement may eventually also increase the effective half-life by favoring rebinding of TCR to MHC peptide complexes when they chemically "dissociate." Qi *et al*. (6) suggest a new advantage of keeping the TCR and MHC peptide small and rigid. The more rigid molecular structures and internal subdomains may lead to a higher transition state energy that can increase the half-life without changing the affinity. Molecules with longer tethers would have more access to the easy reorientation processes that would minimize the differences between solution and synaptic interactions. If half-life is the critical parameter that determines signaling, then both confinement effects leading to rebinding and changes in the transition states that lead to more intrinsically stable complexes will both contribute to enhancing signaling. Thus, the short arms of the TCR and MHC increase the impact of a firm, rigid handshake on the quality the TCR uses to transduce a signal: the 2D half-life.

Future Prospects

Qi *et al*. (6) did not perform detailed calculations to determine the order of magnitude of 3D to 2D half-life conversion. There is insufficient information about interactions in synapse to make such a calculation. Existing data suggest that a universal conversion factor may not exist. For example, although having no impact to 3D affinity, reducing flexibility by shortening the length and randomizing the orientation of some receptor–ligand molecules has been found to decrease 2D affinity by reducing 2D on-rate but not 2D off-rate (17). Disrupting the smoothness and continuity of the confinement zone by surface roughness has been shown to reduce the effective 2D affinity (18). Conformational changes in the binding site have been shown to result in different changes in the 2D and 3D on-rates and off-rates (19). These data emphasize the need for direct experimental measurements. There are two important areas where measurements are needed. It would be valuable to have more measurements of solution half-life and thermodynamic properties for different adhesion/synaptic interactions in which the same structural scaffold is used for different functional interactions. Examples include many Ig family adhesion molecules, the C-type lectin natural killer inhibiting and activating receptors, and the integrins. Equally critical will be the direct measurements of half-lives in adhesive interfaces. Two approaches that are currently tractable are the use of supported planar bilayers to visualize interactions (20) and erythrocyte-based interaction sensors (17), but moving these measurements into native cell–cell interfaces would also be an important challenge for the future.

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