Mechanisms for T cell receptor triggering

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Abstract | There is considerable controversy about the mechanism of T cell receptor (TCR) triggering, the process by which the TCR tranduces signals across the plasma membrane after binding to its ligand (an agonist peptide complexed with an MHC molecule). Three main types of mechanism have been proposed, which involve aggregation, conformational change and segregation. Here, we review recently published evidence for each type of mechanism and conclude that all three may be involved. This complexity may reflect the uniquely demanding nature of TCR-mediated antigen recognition, which requires the detection of a very weak 'signal' (very rare foreign peptide–MHC ligands) in the presence of considerable 'noise' (abundant self peptide–MHC molecules).

Immunoreceptor tyrosine-based activation motif
(ITAM). A sequence that is present in the cytoplasmic domains of the invariant chains of various cell-surface immune receptors, such as the T cell receptor–CD3 complex. Following phosphorylation of their tyrosine residue, ITAMs function as docking sites for Src homology 2 (SH2) domain-containing tyrosine kinases and adaptor molecules, thereby facilitating intracellular signalling cascades.

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The T cell receptor (TCR) consists of a variable TCRαβ heterodimer that binds to ligands. The TCR forms a multisubunit receptor complex with the non-variable signal transduction CD3 complex, which contains CD3γ, CD3δ, CD3ε and TCRζ subunits. All CD3 complex subunits contain immunoreceptor tyrosine-based activation motifs (ITAMs) in their cytoplasmic domains. TCRαβ binds complexes of peptide and MHC molecules on the surface of antigen-presenting cells (APCs) or target cells, which results in biochemical changes in the cytoplasmic portions of the CD3 complex. One biochemical change that is known to be essential for TCR signalling is phosphorylation of the ITAMs in the CD3 complex by the Src family tyrosine kinases LCK and FYN (reviewed in REFS 1,2). In addition, various conformational changes have been reported, some of which are independent of tyrosine phosphorylation[3–5], and some of which are postulated to precede, and be required for, ITAM phosphorylation[6,7]. The process by which TCR binding to peptide–MHC molecules leads to biochemical changes in the cytoplasmic regions of the CD3 complex is referred to as TCR triggering and is the main focus of this Review. Although this area has been reviewed previously, there have been considerable advances made in the past 2 years, and this Review focuses on this new research.

The challenges

While evaluating possible models of TCR triggering, it is important to bear in mind some of the unusual features of TCR antigen recognition, which distinguish it from other cell-surface receptor recognition events. These features clarify the challenges faced by any triggering mechanism and place constraints on the types of model that are mechanistically plausible.

Sensitivity. The first notable feature is the very low abundance of agonist peptide–MHC ligands on APCs or target cells. The TCR on a given T cell is 'restricted' to a subset of the MHC molecules on an APC, and the high-affinity agonist (typically 'foreign') peptide will only be present on a tiny fraction of these molecules. Hence TCR triggering needs to be very sensitive, and indeed TCRs can be triggered when only a single antigenic peptide–MHC ligand is within the contact area[8–10].

Discrimination. A second feature is the presence on APCs and target cells of abundant self peptide–MHC molecules to which the TCR can also bind. To develop into mature peripheral T cells, developing T cells need to undergo positive selection whereby only T cells expressing TCRs that bind self peptide–MHC molecules can survive. Therefore, all peripheral T cells express TCRs that bind self peptide–MHC molecules. Indeed, there is evidence that continued recognition of self peptide–MHC molecules is required for survival of peripheral T cells[11–13]. Although negative selection of developing T cells ensures that the TCRs do not have a high affinity for self peptide–MHC molecules, the affinity threshold for negative selection is sharp and close to the affinity threshold for foreign peptide–MHC ligand recognition[14]. As a result, any given TCR is likely to bind a fraction of self peptide–MHC molecules with affinities that are not much lower than the affinities for foreign peptide–MHC ligands. Furthermore, the self

Serial-triggering model
A model that was proposed to account for the observation that small numbers of agonist peptide–MHC complexes seemed to trigger large numbers of T cell receptors (TCRs), and that postulates that a given peptide–MHC complex can serially bind to and trigger multiple TCRs. As it is the number of productive TCR engagements that determines peptide–MHC efficacy, high-affinity peptide–MHC complexes with long half-lives may be less effective. According to this model there is an optimal affinity or half-life for a TCR–peptide–MHC complex.

peptide–MHC molecules that a given TCR can bind are collectively likely to be far more abundant than foreign agonist peptide–MHC ligands, only a few of which will be recognized by a specific TCR. Because T cells are sensitive to only a small number of recognition events, the stochastic nature of the molecular events involved creates a serious problem of discrimination (BOX 1): how do T cells detect very low numbers of foreign peptide–MHC ligands (signal) in the presence of high levels of self peptide–MHC molecules (noise)?

Molecular mechanisms that have been proposed to explain this remarkable ability to discriminate signal from noise either extend the time available for recognition or invoke cooperative effects, whereby individual TCRs somehow communicate with each other following ligand recognition. Given the remarkable speed of antigen recognition by T cells[15], only cooperative effects can satisfactorily account for discrimination[16]. This cooperation could either involve a direct physical interaction, which would require the TCR–CD3 complexes to form aggregates[17,18], or communication via signalling pathways, a process termed signal spreading[16,19,20].

Versatility. A third requirement of TCR recognition is that it is necessary for the same TCR to recognize multiple ligands with a range of affinities and produce different responses depending on the affinity. For example, in the thymus, developing T cells need to recognize both low-affinity and high-affinity self peptide–MHC molecules and respond differently to each[21]. Similarly, mature T cells require recognition of low-affinity self peptide–MHC molecules to promote survival, whereas recognition of high-affinity foreign peptide–MHC ligands leads to activation and proliferation. Any triggering mechanism or mechanisms must allow recognition of a range of different ligands and produce distinct signals depending on the strength of binding.

Structural diversity. A fourth unusual feature of TCR recognition is the diverse nature of the binding interface between the TCR and a peptide–MHC molecule, and the fact that TCRs need to recognize ligands (foreign peptide) that they have never previously encountered. Although there are clearly common general features in TCR–peptide–MHC complex interactions, the variability of peptide sequences and TCR complementarity-determining region 3 (CDR3) loops results in great diversity in the fine structure of the interface. There are conserved contacts between subsets of TCRs (with similar variable (V) segments) and subsets of MHC molecules[22,23], but no contacts or conformational changes at the binding interface have been identified that are conserved in all TCR–peptide–MHC complex structures[22–24]. Rossjohn *et al.*[25] observed that a triad of MHC class I residues was involved in all published structures of TCR–peptide–MHC class I complexes, but their TCR contacts were variable, as was their contribution to binding energy[26]. Thus, despite having conserved features, such as a broadly diagonal orientation, the TCR–peptide–MHC binding interface is diverse at the atomic level, with no contacts or conformational changes that are common to all TCR–peptide–MHC complexes. Any triggering mechanism needs to accommodate this structural diversity.

The key issue in TCR triggering is explaining how TCR binding to a peptide–MHC complex results in biochemical changes in the cytoplasmic domains of the TCR–CD3 complex. Models of TCR triggering invoke one or more of three basic mechanisms: aggregation, conformational change and segregation or redistribution of the TCR–CD3 complex[27]. Although it is likely that triggering will involve a combination of these mechanisms, it is useful to consider each mechanism separately. Several TCR signalling models (such as the serial-triggering model[28] and the kinetic proof-reading model[29,30]) have been proposed to account for certain quantitative features of T cell activation such as antigen discrimination. As they do not imply any particular molecular mechanisms of TCR triggering, they are not discussed further here.

Aggregation

It is not difficult to envisage how aggregation of TCR–CD3 complexes following TCR engagement could lead to enhanced phosphorylation. This aggregation could, for example, increase the proximity of associated LCK molecules, resulting in the activation of the second receptor in the aggregate by *trans*-autophosphorylation[31]. Forced aggregation of TCRs using either soluble antibodies or soluble multimeric forms of peptide–MHC complexes is sufficient to initiate TCR triggering. Although this clearly shows that artificial aggregation is sufficient for

Box 1 | **Distinguishing signal from noise**

T cells can detect tiny amounts of high-affinity foreign peptide–MHC ligand (signal) presented in the context of large amounts of low-affinity self peptide–MHC complex (noise). To appreciate this feat, it is helpful to consider a specific example. Consider a T cell receptor (TCR) that dissociates from a self peptide–MHC complex with an average half-life of 1 second ($k_{off} = 0.69\ s^{-1}$) and from a foreign peptide–MHC complex with a half-life of 5 seconds ($k_{off} = 0.14\ s^{-1}$), but has the same on rate for both ($k_{on} = 0.001\ \mu m^2\ s^{-1}$). Further assume that the concentration of the self peptide–MHC ligand is tenfold higher than that of the foreign peptide–MHC ligand ($10\ \mu m^{-2}$ and $1\ \mu m^{-2}$, respectively). How can the T cell only respond when foreign peptide–MHC complexes are present?

The simplest discrimination mechanism is the number of occupied TCRs. With some assumptions (such as the conservation of TCR and peptide–MHC complexes) we can compute the number of engaged TCR complexes (C) using the following equation:

$$C = \frac{A}{2}\left[P_T + T_T + K_d - \sqrt{(P_T + T_T + K_d)^2 - 4P_T T_T}\right]$$

where A ($\pi 5^2\ \mu m^2$) is the area of the contact interface, P_T and T_T are the total concentration of the peptide–MHC and TCR complexes, respectively, and K_d is the dissociation constant. Substituting in the values above, we find that TCR occupancy produces a poor signal-to-noise ratio of 0.33 ($0.42\ \mu m^{-2}$ / $1.2\ \mu m^{-2}$). Therefore, on average, more receptors will be occupied by self peptide–MHC ligand than foreign peptide–MHC ligand.

An alternative output that the T cell can potentially respond to is the rate of TCR binding events, as in the serial-triggering model[28]. The rate of binding events is simply $k_{off}C$, which is $68\ s^{-1}$ and $4.5\ s^{-1}$ for self and foreign peptide–MHC ligands, respectively. This produces an even lower signal to noise ratio of 0.07. This ratio can be improved if we stipulate that each TCR will only signal if bound for a threshold time (τ), as in the kinetic proof-reading model[29,30]. In this case, the rate of productive binding events is $k_{off}C\exp(-k_{off}\tau)$. Taking $\tau = 5$ seconds, we find that the revised binding rate is $2.1\ s^{-1}$ and $2.2\ s^{-1}$ for self and foreign peptide–MHC ligands, respectively, which corresponds to a signal-to-noise ratio of 1.1. Although this is a substantial improvement, it is still inadequate because both self and foreign peptide–MHC ligands will produce a similar number of productive binding events, making them indistinguishable.

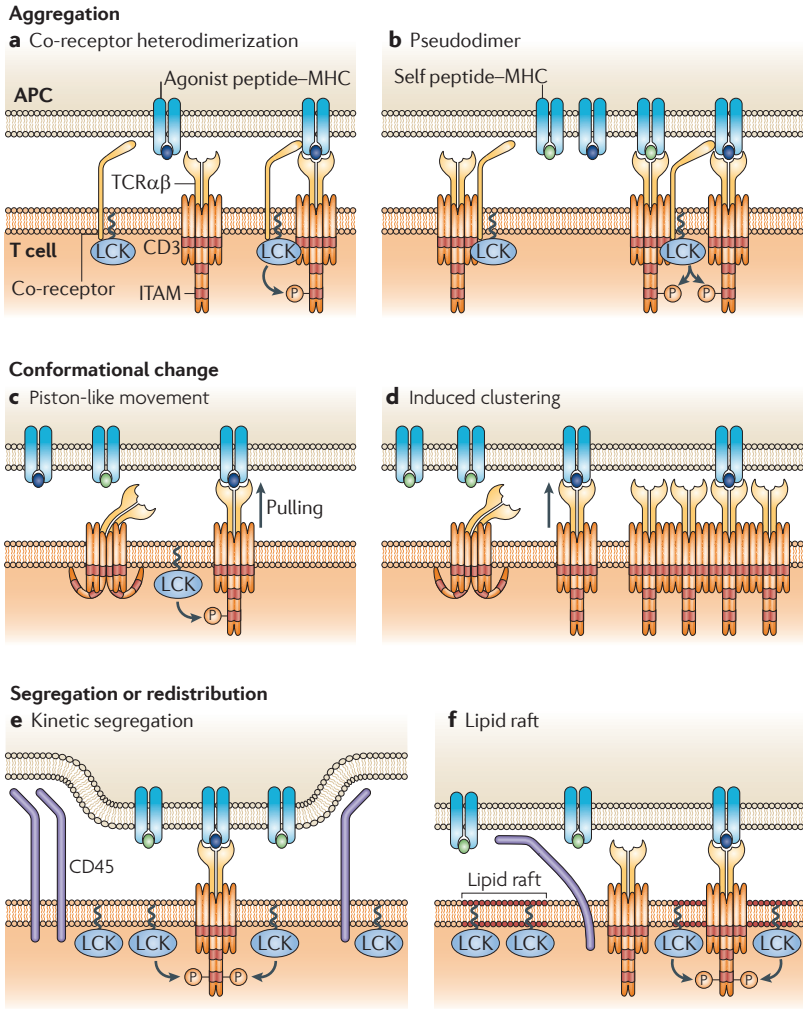

Figure 1 | **Mechanisms of TCR triggering.** Models are grouped according to whether the primary mechanism is aggregation, conformational change or segregation. **a** | In the co-receptor heterodimerization model, co-receptor binding to the same peptide–MHC complex as the T cell receptor (TCR) brings co-receptor-associated LCK into proximity with TCR–CD3 immunoreceptor tyrosine-based activation motifs (ITAMs). **b** | The pseudodimer model postulates that two TCRs are brought together by binding low-affinity self (green) or high-affinity agonist (blue) peptide–MHC ligand and that the co-receptor associated with one TCR engages the agonist peptide–MHC complex, thereby forming a dimer. **c** | A piston-like displacement of the TCR–CD3 complex is induced by the mechanical effects (primarily pulling; black arrow) of peptide–MHC binding to the TCR. This leads to a change in the conformation of the CD3 cytoplasmic domains, allowing ITAM phosphorylation. **d** | Clustering is induced by a conformational change in TCR–CD3 complex, possibly enhancing kinase activity. The conformational change in the cytoplasmic domains is depicted as dissociation from the membrane, as proposed in the 'safety-catch' model[7]. **e** | The kinetic-segregation model proposes that TCR binding to peptide–MHC ligand traps the TCR–CD3 complex in close-contact zones, thereby segregating it from the inhibitory tyrosine phosphatase CD45, leading to stable phosphorylation of TCR–CD3 ITAMs by LCK. **f** | Lipid raft models postulate that peptide–MHC engagement somehow results in partitioning of the TCR–CD3 complex into regions of membrane enriched in LCK and deficient in CD45. APC, antigen-presenting cell.

triggering, the challenge has been to explain how binding of agonist peptide–MHC ligand alone can induce aggregation when the ligand is present at such low surface densities. Indeed, TCR triggering can be observed with a single agonist peptide–MHC ligand[8–10]. Several models have been proposed to account for this.

The co-receptor heterodimerization model (reviewed in REF. 32) postulates that CD4 or CD8 co-receptors bind to the same agonist peptide–MHC complex as the TCR, thereby recruiting co-receptor-associated LCK into close proximity with CD3 complex ITAMs to mediate their phosphorylation (FIG. 1a). However, TCR triggering can occur in the complete absence of co-receptors[33,34], indicating that co-receptor heterodimerization is not essential for TCR triggering. Furthermore, in most studies, soluble agonist peptide–MHC monomers cannot induce TCR triggering, indicating that co-receptor heterodimerization is usually not sufficient for TCR triggering.

The pseudodimer model postulates a role for self peptide–MHC molecules in TCR triggering[9,35]. According to this model, one TCR binds an agonist peptide–MHC molecule and a second TCR binds a self peptide–MHC molecule. Dimerization is enhanced because the co-receptor associated with the TCR that is complexed with the self peptide–MHC molecule binds to the agonist peptide–MHC complex. A pseudodimer is hence formed by the dual interaction of a second TCR with self peptide–MHC and its associated CD4 or CD8 co-receptor with the agonist peptide–MHC complex (FIG. 1b). As self peptide–MHC molecules are present at a much higher surface density, this helps to address the problem of low surface density of agonist peptide–MHC molecules. A key prediction of this model is that self peptide–MHC molecules would enhance TCR triggering, especially at low densities of agonist peptide–MHC ligands. Evidence for this is clearest in the case of $CD4^+$ T cells, whereas there are conflicting data for $CD8^+$ T cells (reviewed in REF. 36). Interestingly, a high proportion of self peptide–MHC molecules (50–100% of those tested) seem to enhance agonist peptide–MHC ligand recognition[36,37]. It is therefore plausible that aggregates of agonist and self peptide–MHC molecules could induce clustering of TCRs and co-receptors simply by binding to the TCR.

Finally, some models propose that engagement of peptide–MHC molecules induces conformational changes in the TCR–CD3 complex that predispose it to dimerization and aggregation[38,39]. These models are considered in the next section.

There is some controversy as to the natural state of the TCR at the cell surface. Some studies report that at least a proportion of TCRs are in aggregates (or clusters)[40–43] that can form what some have termed 'protein islands'[41], whereas other studies suggest that the TCRs are primarily monomeric[44,45]. The observed TCR clusters were 30–300 nm in diameter and contained 5–20 TCRs[41–43]. It is noteworthy that in the cases in which clustering was reported, the T cells were in contact with artificial surfaces and the clusters were observed in these contact areas. These results can be reconciled if it is postulated that TCRs are primarily monomeric but are predisposed to clustering following initial triggering, and that contact with surfaces can induce clustering by inducing weak TCR triggering[46].

Whatever the natural state of the TCR before engagement of peptide–MHC molecules, it is clear that this engagement results in increased aggregation of TCRs into what have been termed 'microclusters', containing 10–100 TCRs[43,47]. The formation of these microclusters was first visualized by total internal reflection fluorescence (TIRF) microscopy of T cells in contact with planar bilayers[47,48]. Interestingly, their size seems to vary little with the surface density of peptide–MHC molecules[43].

This raises the question as to the mechanism of microcluster formation. Is it simply a physical consequence of the binding of agonist and self peptide–MHC molecules by TCRs and their co-receptors? Or are other processes involved? In support of the latter is the observation that microcluster formation is blocked by the inhibition of actin polymerization, suggesting a role for the actin cytoskeleton in microcluster formation[47,48]. It is possible that TCR triggering itself leads to clustering, through, for example, signalling molecules that associate with and crosslink the TCR–CD3 cytoplasmic domains. Imaging studies to date have been unable to resolve whether TCR triggering precedes microcluster formation or vice versa. However, some formation of microclusters is still observed in the presence of Src tyrosine kinase inhibitors[48,49]. This shows that ITAM phosphorylation is not required for microcluster formation, but does not rule out a role for other signalling pathways.

In conclusion, recent studies on the ability of self peptide–MHC molecules to engage TCRs suggest that aggregation is a plausible mechanism of TCR triggering despite the low density of agonist peptide–MHC ligands.

Conformational change

Several models have been proposed that invoke binding-induced conformational change as a mechanism of TCR triggering. An attraction of these models has been that they can account for triggering at very low densities of agonist peptide–MHC molecules. A conformational change model needs to explain in molecular detail how TCR binding to peptide–MHC molecules can lead to changes in the CD3 cytoplasmic domains. At present there are no models that can do this satisfactorily. This is partially because we still do not know the structure of the intact TCR–CD3 complex. However, structural and other studies of different portions of the TCR–CD3 complex have provided intriguing clues.

TCR ectodomains. Although the binding of peptide–MHC molecules often induces conformational changes in the TCR[24], these are primarily in the contact area and are not widely conserved. However, crystallographic studies of a TCR in the bound and unbound state suggested a possible subtle conformational change in the membrane-proximal AB loop of the TCRα constant (Cα) domain[50]. A recent follow-up study[51] confirmed this using a different technique and reported a similar change in a different TCR. In addition, this study showed that mutation of residues in the AB loop abrogates TCR antigen recognition[51]. However, the AB loop of the TCR Cα domain is often poorly resolved in the crystal structures of TCR–peptide–MHC complexes, so it is not clear how widespread the conformational change is following TCR binding[51]. Further structural studies in which the relevant TCR regions are well resolved are needed to address this.

How could such a conserved conformational change in the TCR Cα domain be produced given the diversity at the binding interface of the TCR–peptide–MHC complex? One possible mechanism, discussed below, is through a mechanical force generated by the peptide–MHC complex pulling on the TCR. Another question is how changes in this region of the TCR could be transduced to the cell interior. Based on their observation that mutations of the AB loop affect TCR dimerization, Kuhns *et al.*[40] have proposed that the conformational change in the AB loop of the TCR Cα domain regulates TCR dimerization. Thus, binding-induced conformational changes may signal by inducing clustering. Similar models have been previously proposed based on reports that TCR binding to peptide–MHC complexes leads to clustering[38,39].

CD3 ectodomains. The ectodomains of CD3δ and CD3γ form stable heterodimers with the CD3ε ectodomain, and the structures of the CD3δε[52,53] and CD3γε[54,55] heterodimers have been determined. Although it is not yet understood how the TCRαβ ectodomain associates with the CD3 ectodomains, compelling evidence of direct association between the transmembrane regions[56] suggests that these ectodomains will be in close physical contact, at least in the portions that are close to the membrane. In the absence of structural data, indirect methods have been used to infer the arrangement of the TCR and CD3 ectodomains, with contradictory results[57]. However, recent studies support a model in which the CD3 ectodomains are arranged on one side of the TCRαβ ectodomain, with CD3δ and CD3γ contacting the TCR Cα and Cβ domains, respectively, and with the two CD3ε subunits in close proximity to each other[40,58,59]. In this model, the AB loop of the TCR Cα domain is not in contact with the CD3 heterodimers and is potentially available to mediate TCR dimerization[40].

Reinherz and colleagues[54] have observed that the CD3γε heterodimer seems to be relatively rigid and, on this basis, argued in favour of a role for mechanical piston-like forces (see below). Structural studies of the CD3γε and CD3δε heterodimers have not provided any direct evidence for conformational change, but molecular dynamic simulations have identified possible conformational changes in the CD3ε stalk[60]. Mutation of residues in this region, including in a conserved CXXC motif, abrogated TCR signalling *in vitro* and T cell development *in vivo*[60,61]. Surprisingly, these mutant forms of CD3ε inhibited TCR function even in the presence of excess wild-type CD3ε[60], supporting a model in which TCR–CD3 complexes form cooperative microclusters[18].

CD3 cytoplasmic domains. Several studies have suggested that the cytoplasmic portions of the CD3 complex undergo conformational change and that this could be implicated in TCR triggering. Alarcon and colleagues[3–5] provided evidence for conformational changes when

Kinetic proof-reading model
A model that was proposed to account for the ability of T cells to discriminate between peptide–MHC ligands that have small differences in their affinity or half-life. It postulates that T cell receptor (TCR) triggering requires multiple sequential steps that can only proceed while the TCR is engaged with a peptide–MHC complex and that are completely reversed as soon as the TCR dissociates from the complex.

the TCR is engaged by antibody or by peptide–MHC molecules, and implicated a proline-rich motif in CD3ε that binds the adaptor protein non-catalytic region of tyrosine kinase (NCK). However, this motif seems to be involved in regulating the expression of TCR and CD3 subunits, and not in TCR triggering[62,63].

Stern and colleagues[6] used circular dichroism to investigate the protein structures and showed that the cytoplasmic portion of TCRζ undergoes a conformational change upon binding to acidic lipid vesicles. This change in conformation is accompanied by changes in tyrosine fluorescence and a decreased susceptibility to phosphorylation. More recently it was shown that a basic residue rich sequence (BRS) in CD3ε mediates binding to negatively charged phospholipids in the cell membrane[7,64]. Wucherpfennig and colleagues[7] also provided nuclear magnetic resonance evidence that the tyrosine residues in the CD3ε ITAMs may be buried in the lipid bilayers. Based on these findings, and the observation that the TCRζ cytoplasmic domain also contains BRSs, a 'safety-catch' model was proposed, postulating that some TCR–CD3 ITAMs are protected from phosphorylation in the resting state (safety on) and that TCR engagement by ligand results in the dissociation of these ITAMs from the phospholipids in the cell membrane (safety off), exposing them to phosphorylation[6,65]. However, the finding that exposure of T cells to the tyrosine phosphatase inhibitor pervanadate induces dramatic TCR–CD3 ITAM phosphorylation[66–69] shows that these ITAMs are accessible to kinases in the absence of TCR engagement. Also arguing against this model is the finding that mutation of the CD3ε BRS abrogates phosphorylation[64,69]. Wucherpfennig and colleagues[70] have countered that the effects of pervanadate are secondary to lipid modification and that BRS mutants could disrupt the CD3ε conformation. Further experiments are needed to resolve these issues.

Mechanical effects. Recently, there has been increased interest in conformational change models that invoke a role for mechanical forces such as pulling or shearing[27,54,71–73]. These models have been inspired by several lines of evidence. First, the recognition that imposition of a mechanical pulling force on the TCR–CD3 complex is an inevitable consequence of binding to the peptide–MHC ligand[74], which has recently been supported by measurement of the TCR–peptide–MHC complex

Box 2 | **Two-dimensional binding properties**

Given that the T cell receptor (TCR) and peptide–MHC complex are normally associated with cell surfaces, it has long been a goal to measure the binding properties of this interaction *in situ*. As membrane-associated molecules are constricted to diffuse in two dimensions, their binding properties are stated in two-dimensional (2D) units, in contrast with solution binding properties, which are three-dimensional (3D). Two recent landmark studies have measured the 2D binding properties using very different approaches, producing intriguing results and valuable insights[105,106]. Huppa *et al.*[106] used fluorescent protein tags, total internal reflection fluorescence (TIRF) microscopy and fluorescent resonance energy transfer (FRET) to measure the surface densities of TCR, peptide–MHC and TCR–peptide–MHC complexes at the interface between T cells and planar bilayers onto which peptide–MHC complexes and other ligands had been attached. By assuming that these interactions were at equilibrium, they used these data to calculate the 2D dissociation constant (K_d) in the T cell–bilayer interface at the resolution of TIRF microscopy. By measuring the duration of FRET for a large number of TCR–peptide–MHC complexes they were also able to estimate the 2D off rate (k_{off}).

Huang *et al.*[105] used mechanical assays to measure the 2D binding parameters. In these assays, peptide–MHC molecules that were attached directly or indirectly to erythrocytes were brought into contact with T cells for varying periods, and binding was detected by deformation of the erythrocytes when the cells were pulled apart. Both studies found that the 2D k_{off} was significantly faster than the solution k_{off}, and that this difference was reduced by inhibitors of the actin cytoskeleton[105,106]. This is evidence that the TCR–peptide–MHC interaction is subjected to mechanical forces, as previously predicted[74]. A second key finding was that the 2D K_d was highly variable, and that this variability was largely the result of changes in the 2D on rate (k_{on}). Huppa *et al.* found considerable variation (~200-fold) for a given TCR–peptide–MHC combination even within a single contact interface[106], whereas Huang *et al.* found even greater variation (~2,000-fold) between a set of peptide–MHC analogues, despite the fact that their solution or 3D k_{on} did not vary much[105]. Interestingly, the dramatic differences in 2D k_{on} correlated well with activation potency[105]. Paradoxically, Huang *et al.* also found that the 2D k_{off} correlated with activation potency and inversely with 3D k_{off}. Furthermore, the 2D k_{on} was reduced by treatments that would be expected to inhibit TCR triggering and TCR clustering, and the same treatments decreased the 2D k_{off}. A plausible explanation for these intriguing results is that TCR triggering, which is induced by binding to agonist peptide–MHC ligand, rapidly leads to changes in the local surface density and/or orientation of the TCR–CD3 complex, and these changes are accompanied by an increase in the mechanical force that the TCR–peptide–MHC interaction is subjected to. Triggering-induced TCR clustering could increase the surface density of TCR–CD3 complexes over 100-fold in the immediate vicinity of the engaged TCR, accounting for most of the apparent increase in 2D k_{on}. Triggering-induced clustering could also enhance the apparent 2D k_{on} by optimizing the TCR–CD3 orientation (for example, by making it more upright). A previous study from the same group showing an increased probability of TCR–peptide–MHC binding upon subsequent encounters also supports triggering-induced enhancement of TCR–CD3 binding by clustering and/or reorientation[107].

These studies reveal that measuring 2D binding properties in the context of functional T cells is complicated by triggering-induced changes in TCR surface density and/or orientation and in the mechanical forces that act on the TCR–peptide–MHC interaction. Measurements in systems in which these effects are controlled will be necessary to fully elucidate the relationship between 2D binding properties and TCR triggering.

off rate in intact cells (BOX 2). Second, the structural evidence that the CD3γε heterodimer may be rigid[54] and that the protruding FG loop of the TCR Cβ domain is in contact with the membrane-distal top end of the CD3γε heterodimer[58,75]. Thus any force applied to the TCRαβ heterodimer could be transmitted through the CD3γε heterodimer to the membrane. Third, the long-standing observation that T cell activation by artificial ligands is optimal when the ligands are anchored to a surface[76,77]. Fourth, an appreciation that elongation of the peptide–MHC ligand could abrogate TCR triggering by reducing any mechanical force on the TCR[71,78,79]. And, finally, reports that the application of mechanical force on the TCR enhances TCR triggering[73,80].

The mechanical forces could be generated by several processes[74]. The small size of the TCR–peptide–MHC complex would generate a force as larger molecules that are excluded from or compressed within close-contact areas try to diffuse in or straighten out. This is consistent with the observation that elongation of the peptide–MHC ligands abrogates triggering[78,79]. Active sources of force include the movement of cell processes and membranes, driven by cytoskeletal and endocytic mechanisms. This is supported by the long-standing observation that reagents that disrupt the actin cytoskeleton abrogate TCR triggering[81].

A major attraction of these mechanical force models is that they can readily explain how binding at a structurally variable peptide–MHC binding site in the TCR could induce the same conformational change in all TCR–CD3 complexes, because the mechanical force is a direct consequence of binding. Discrimination between different ligands comes about because the duration of the applied mechanical force and the resulting conformational change will be determined by the duration of binding.

It has also been proposed that peptide–MHC binding could push and/or twist the TCR[57,82]. However, an advantage of pulling or shearing mechanisms is that they are inherently specific because only specific interactions between the TCR and the peptide–MHC ligand can resist the pulling or shearing forces. By contrast, pushing does not necessarily require binding and could be nonspecific.

How might the effect of mechanical pulling on the TCR–CD3 complex be transduced into the cell interior? One possibility is that binding leads to a piston-like movement of the CD3 cytoplasmic tails, relative to the plasma membrane, that could alter their conformation (FIG. 1c). Evidence cited in support of this includes the apparent rigidity of the CD3 ectodomains[54] and the observations that mutations in the conserved stalk region of CD3ε abrogate T cell activation[60,61]. In addition, the transmembrane regions of the TCR–CD3 complex specifically interact with each other, suggesting that they could form a relatively rigid structure[83,84]. However, convincing direct evidence of rigidity of the entire CD3 complex is lacking, particularly for the segments between the membrane and the ITAM region, making it unclear how piston-like movement could be transduced to the ITAMs.

A second possibility is that pulling induces a conformational change in the structure of the TCR–CD3 ectodomains and/or transmembrane domains that leads to clustering of the engaged TCR–CD3 complex with other TCR–CD3 complexes (FIG. 1d). Such a mechanism has been proposed for B cell receptor (BCR) triggering[85]. Pierce and colleagues[86] have shown that binding of monomeric, surface-immobilized antigen to the IgM BCR induces clustering through its membrane-proximal Cμ4 domain.

In conclusion, evidence that a conformational change is involved in TCR triggering is mounting, but the nature of the conformational change and the mechanism by which peptide–MHC binding induces the conformational change remain unclear.

Segregation and redistribution

A third type of mechanism that has been proposed for TCR triggering is binding-induced segregation or redistribution of the TCR–CD3 complex with respect to other cell membrane-associated proteins. The TCR–CD3 complex is embedded in the plasma membrane along with molecules that favour signalling (such as the tyrosine kinase LCK) and molecules that inhibit signalling (such as the receptor tyrosine phosphatases CD45 and CD148). Treatment of resting T cells with inhibitors of tyrosine phosphatases leads to increased phosphorylation of TCR–CD3 ITAMs, resulting in TCR signalling and T cell activation even in the complete absence of TCR ligands[66–68]. Recently, Acuto and colleagues[87] reported that a substantial amount of LCK is constitutively active in resting T cells and that this did not increase detectably upon TCR engagement. These results suggest that phosphorylation by constitutively active LCK is held in check by constitutively active phosphatases. It follows that anything that disturbs this balance could induce an increase in TCR–CD3 ITAM phosphorylation and TCR signalling. One way of disturbing this balance is through the redistribution of the TCR–CD3 complex, LCK and CD45 with respect to each other.

Two types of redistribution or segregation models have been proposed. The kinetic-segregation model proposes that segregation of an engaged TCR–CD3 complex from CD45 is driven by differences in ectodomain size[88,89], whereas the lipid raft model proposes that redistribution is driven by changes in the lipid environment of the TCR[90].

As the kinetic-segregation model has recently been reviewed[91], it is discussed only briefly here. The model postulates that multiple zones of close contact (~15 nm apart) form at the cell–cell interface, from which molecules with large ectodomains, such as the inhibitory tyrosine phosphatases CD45 and CD148, are excluded (FIG. 1e). As a result, ITAM phosphorylation is strongly favoured. Binding to an agonist peptide–MHC ligand serves to trap the TCR–CD3 complex within a close-contact zone where it is protected from dephosphorylation by CD45 and CD148. This results in long-lived phosphorylation of TCR–CD3 ITAMs, leading to recruitment and activation of ζ-chain-associated protein kinase of 70 kDa (ZAP70) and subsequent phosphorylation by ZAP70 of the adaptor molecules SH2 domain-containing leukocyte protein of 76 kDa (SLP76; also known as LCP2) and linker for activation of T cells (LAT).

Lipid raft
An area of the plasma membrane that is rich in cholesterol, glycosphingolipids, glycosylphosphatidylinositol-anchored proteins and several signalling proteins — such as Src family kinases, LAT (linker for activation of T cells) and PAG (protein associated with glycolipid-enriched microdomains). These domains are also known as glycolipid-enriched microdomains (GEMs) and detergent-insoluble glycolipid-enriched membranes (DIGs).

Several lines of evidence support this model: first, CD45 and CD148 are excluded from areas of TCR triggering[43,92]; second, truncation of the large CD45 and CD148 ectodomains inhibits TCR triggering[92,93]; third, elongation of the peptide–MHC complex inhibits TCR triggering[78,79]; fourth, surface-associated TCR ligands induce TCR triggering more effectively than their soluble counterparts[76,77]; and, finally, recognition by engineered TCRs is optimal when the epitope is positioned close to the plasma membrane of the target cell[94,95]. Although these results provide compelling evidence in support of the kinetic-segregation model, they do not exclude other mechanisms. Indeed, the last three lines of evidence cited above are also consistent with a mechanical pulling mechanism for TCR triggering.

Lipid raft models propose that TCR binding to peptide–MHC complexes leads to an association of the TCR–CD3 complex with lipid rafts. This enhances phosphorylation of TCR–CD3 ITAMs because lipid rafts are enriched in some proteins (such as LCK) and deficient in others (such as CD45) (FIG. 1f). The binding of peptide–MHC to TCR–CD3 may alter its lipid environment by immobilizing and/or clustering TCR–CD3 complexes. The role of lipid rafts has been controversial because the early techniques that were used to implicate them were later found to be unreliable[96]. Recent studies using new techniques have confirmed the existence of these structures and showed that they are smaller and more dynamic than previously appreciated[97]. Although there is substantial evidence supporting a role for lipid rafts in TCR signal transduction[90,98–100], some of this evidence has been challenged[101–104]. One study was unable to show co-localization of lipid raft markers with TCR microclusters[102]. Although this argues against a role for lipid rafts in microcluster formation it does not rule out a role for lipid rafts at earlier time points and/or on a smaller scale. Further studies using higher resolution imaging techniques are needed to elucidate the role of lipid rafts in the initial steps of TCR triggering. Although these findings suggest that segregation or redistribution of the TCR are likely to have an important role in TCR triggering, it is unclear whether they alone are sufficient to induce triggering.

Conclusion

Is it possible to reconcile all of the diverse experimental findings that support the different triggering mechanisms discussed above? We suggest the following scenario (FIG. 2). In resting T cells there is a delicate balance between tyrosine phosphorylation of the TCR–CD3 complex by constitutively active LCK and dephosphorylation by constitutively active phosphatases, with the phosphatases dominating (FIG. 2a). In principle, TCR triggering can be induced by any mechanism that tilts this balance in favour of phosphorylation. Engagement of the peptide–MHC complex leads simultaneously to aggregation of TCR–CD3 complexes and their segregation from other membrane proteins, which decreases the local phosphatase activity and increases the local kinase activity of LCK (FIG. 2b). Engagement also leads to conformational change in the cytoplasmic domain of the

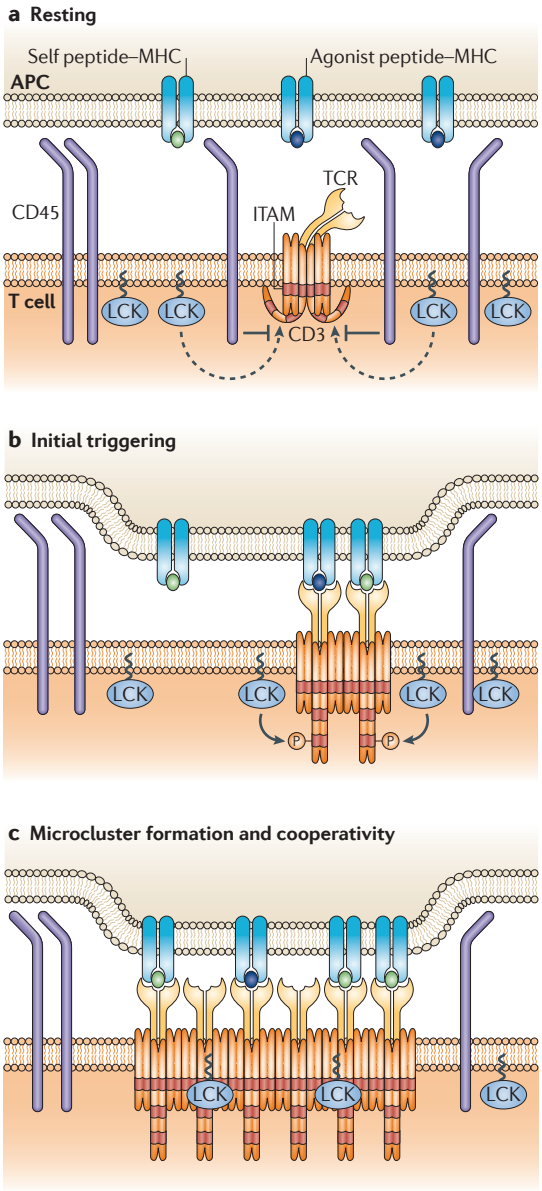

Figure 2 | **Integrated TCR triggering model. a** | In the resting state, the T cell receptor (TCR)–CD3 complex is primarily monomeric or forms very transient small aggregates. Phosphatase activity dominates and the level of TCR–CD3 immunoreceptor tyrosine-based activation motif (ITAM) phosphorylation is low. **b** | TCR engagement with peptide–MHC complex leads to segregation of the TCR–CD3 complex from phosphatases such as CD45, as well as aggregation and conformational change in the TCR–CD3 cytoplasmic domains. It is possible that aggregation alters the lipid environment of the TCR–CD3 complex (not shown). The overall result is a substantial increase in kinase activity and decrease in phosphatase activity, leading to increased ITAM phosphorylation. **c** | The TCR–CD3 complex aggregates enlarge to form microclusters as signalling proceeds. We suggest that these microclusters are the site of cooperative interactions that are required for ligand discrimination; for example, TCRs engaging agonist peptide–MHC ligand enhance signalling by other TCRs in the microcluster through positive feedback. APC, antigen-presenting cell.

TCR–CD3 complex, which may alter susceptibility to phosphorylation. With continued triggering, TCR–CD3 complex aggregates enlarge to form microclusters containing 10–100 TCRs, some of which are bound to foreign peptide–MHC complexes (FIG. 2c).

The crucial requirement for TCRs to discriminate between abundant self and rare foreign peptide–MHC complexes is achieved at two levels. Individual TCRs use mechanisms such as kinetic proof-reading to discriminate binding events of different duration. However, as this is insufficient to cope with the stochastic nature of individual TCR–peptide–MHC interactions, we suggest that TCR microclusters are sites where multiple TCR–CD3 complexes cooperate to enable discrimination of rare foreign peptide–MHC ligands from more abundant self peptide–MHC molecules.

Further progress in our understanding of TCR triggering will require better information about the structure of the full TCR–CD3 complex and how this structure changes on peptide–MHC binding. Determining the structure of transmembrane proteins, particularly multisubunit proteins, is a major research bottleneck and progress in this area is likely to be slow and require considerable ingenuity. Also important is high-resolution information about the arrangement of the TCR–CD3 complex and other cell-surface and signalling molecules and how this arrangement changes following peptide–MHC engagement. Recent developments in super-resolution fluorescence microscopy suggest that advances in this area are probable over the next decade as these new techniques become more widely available.

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Competing interests statement
The authors declare no competing financial interests.

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