# Twisting tails exposed: the evidence for TCR conformational change

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**The mechanism by which the ligand occupancy state of the T cell receptor complex is converted into intracellular signaling information has been a controversial topic. Although the majority of structural studies argue against a conformational change, recent studies support the possibility for such a change within the CD3 components of the TCR complex. In this commentary, the evidence for TCR conformational change is reviewed and potential mechanisms for its initiation are explored.**

Although a great deal is known about the details of TCR signaling, how it is initiated continues to be a topic of intense debate. The TCR complex consists of the αβ heterodimer, the associated CD3 chains (dimers of δε and γε), and the ζ homodimer. The αβ chains engage antigenic peptides bound to the major histocompatibility complex (pMHC), whereas the CD3 and ζ chains function as the signaling components through their immunoreceptor tyrosine-based activation motifs (ITAMs) located in their cytoplasmic domains. The structure of the TCR complex requires that the ligand-binding components somehow communicate to the signaling chains on the cytoplasmic side of the membrane upon appropriate interaction with pMHC.

Many models have been proposed for how TCR signaling is initiated, including TCR oligomerization and conformational change. The oligomerization model has received much support based on the ability of anti-TCR antibodies to cross-link and thereby stimulate T cells (1). Furthermore, T cell activation can be mimicked by antibody treatment of chimeric T cell receptors that contain extracellular domains of other molecules linked to CD3 or ζ-containing ITAM sequences, suggesting that clustering of the molecules is sufficient for triggering activation. However, this clustering model does not account for the great sensitivity of the TCR complex. For instance, it has been shown that a small number of peptides, even one pMHC complex, can initiate signaling events (2). Under these circumstances, conformational change in the TCR is a more attractive model. Conformational change has also been proposed to explain early studies of TCR signaling where receptor multimerization or the avidity of antibodies for the receptor do not sufficiently explain the biochemical results (3). For example, analyses of panels of antibodies against the TCR demonstrated that the ability of the antibody to induce TCR signal transduction or activation was epitope dependent (4, 5). Neither avidity nor cross-linking appeared to fully account for the differential stimulatory abilities of the antibodies, thereby lending support for a role for conformational change.

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## Conformational change within the αβ heterodimer

In general, analyses of crystal structures of TCR–pMHC complexes have argued against the idea of a large conformational change within the αβ chains upon ligand engagement. The conformational change that has been deduced from ligand-bound and ligand-free receptors occurs within the complementarity-determining regions (CDRs) of the TCR, which associate with pMHC, but not in the distal portions of the heterodimer which are adjacent to the cell membrane (6). The movement in the binding site supports an "induced fit" model of TCR–pMHC engagement where adjustments in the CDR loops are made to accommodate the peptide presented by MHC, but these adjustments do not appear to be transmitted to the associated CD3 and ζ molecules (6, 7). However, it is important to note the limitations of the available structural data. Currently, only the ectodomains of the TCR components have been studied in isolation. It is well documented that the transmembrane regions of the TCR are essential for proper TCR assembly (8), and thus the CD3 chains may influence or constrain the structural arrangement of the αβ heterodimer. The conformation of the receptor based on crystal structures cannot be appreciated until the entire TCR complex is studied as one functional unit.

Although the majority of the available structural data does not support a conformational change model, recent data complicates the debate. Interestingly, the crystal structure of the LC13 TCR with its ligand, an Epstein-Barr virus peptide–MHC molecule, compared with the unligated receptor, reveals that a large conformational change occurs in the constant domain of TCRα (9). However, it is unknown how applicable this structural change is to other TCRs.

## Conformational change within the CD3 and ζ chains

Despite the relative lack of consistent data regarding conformational change within the αβ heterodimer, there is increasing evidence that conformational change does occur within the associated CD3 chains of the TCR. This idea is intriguing from a signaling perspective because the ITAMs in the cytoplasmic domains of these components

are the proximal targets of tyrosine phosphorylation. Once they are doubly phosphorylated by the Src family kinase Lck, the Syk tyrosine kinase ZAP-70 is recruited and then promotes the downstream signaling cascade (10). Therefore, alterations in the cytoplasmic tails of the CD3 chains could dramatically affect the availability of the ITAMs and other domains and thus regulate signaling initiation.

The structural regulation of the signaling domains was first proposed for the ζ chain, whose cytoplasmic tail was shown to be differentially available to phosphorylation by Src family kinases based on local environment (11). In the presence of lipids, the cytoplasmic domain of the ζ chain was folded and incapable of being phosphorylated, whereas in aqueous solution the domain was unstructured and readily phosphorylated (Fig. 1 A). Although there are no cellular data available supporting these findings, it was suggested that TCR clustering leads to an increase in local ζ concentration and, consequently, competition for membrane binding, thereby resulting in the release of the cytoplasmic domains of ζ from the membrane. The cytoplasmic domains could also be rearranged by some other mechanism that is not clustering dependent as described later.

More recently, the conformational change debate has shifted its focus to the cytoplasmic tail of CD3ε. In 2002, Alarcón and colleagues reported that upon engagement with certain anti-TCR antibodies or anti-CD3ε antibodies the proline-rich sequence in the cytoplasmic tail of CD3ε becomes accessible, and this was interpreted to reflect a conformational change (reference 12; Fig. 1 B). The exposure of the proline-rich sequence was detected by the ability of CD3ε to inducibly bind the SH3.1 domain of the adaptor protein Nck. Nck was also recruited to the TCR upon antigen treatment of TCR transgenic T cells. The exposure of the motif occurred independently of and before any tyrosine phosphorylation, thereby suggesting that a TCR conformational change preceded TCR signaling. In addition, lateral movement within membranes was not required since the CD3ε proline-rich motif was exposed when cells were stimulated on ice. Perhaps most interestingly, this putative conformational change was not observed with all antibodies used for stimulation. Moreover, a monovalent Fab fragment was able to expose the CD3ε motif, suggesting that the TCR cross-linking did not induce the alteration in the cytoplasmic domain. Although their data convincingly demonstrated that the proline-rich sequence is differentially exposed upon ligand treatment, the specific events leading to this accessibility of the CD3ε sequence, the role of Nck itself, and the biological relevance of the conformational change remain unclear. Overexpression of the SH3.1 domain of Nck appeared to act as a dominant negative by inhibiting complete TCR activation and proper cytoskeleton rearrangement. But clearly such overexpression is subject to significant artifacts as there may be many targets of this domain, and the binding of this region to the CD3ε sequence could disrupt other relevant interactions. Nonetheless, exposure of the motif suggests previously unappreciated movements in the TCR complex.

In this issue, Palmer and colleagues extend the study of CD3ε conformational change to murine thymocytes and peptide ligands (13). In ex vivo studies of OT-I TCR transgenic $CD4^+$ $CD8^+$ thymocytes, both positively and negatively selecting peptides expose the proline-rich region in CD3ε. The conformational change occurred independently of enzymatic and cellular activity as was seen for Jurkat T cells (12). In addition, Gil et al. impressively demonstrate the exposure of the proline-

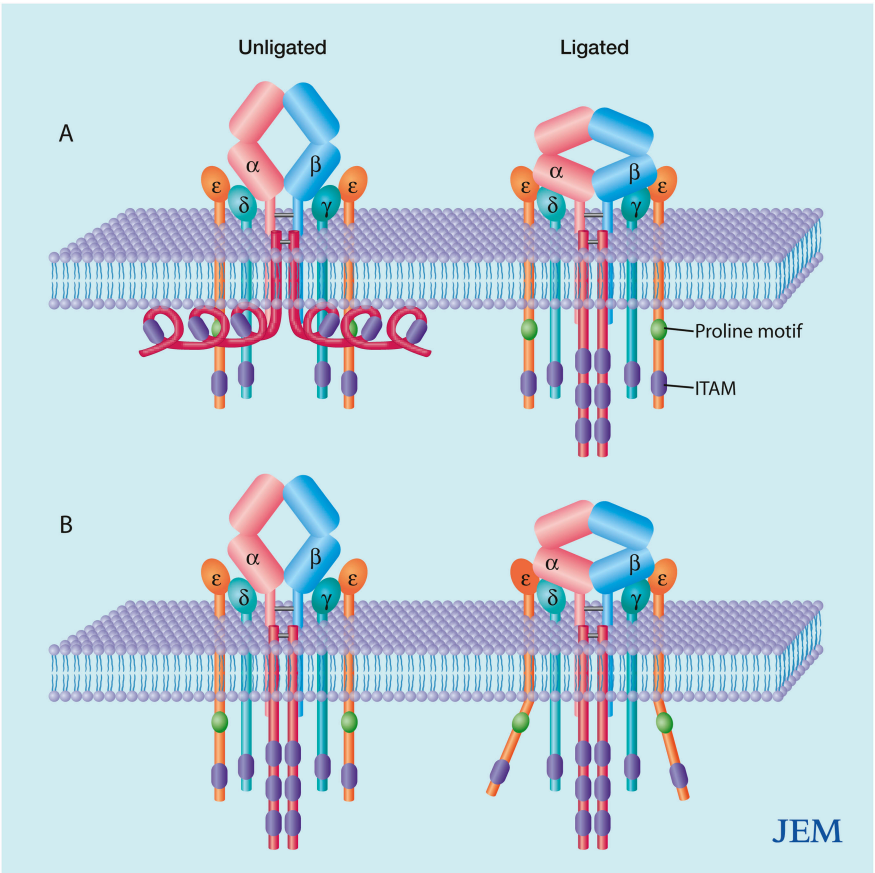


**Figure 1. Potential mechanism for induced exposure of the proline-rich motif in CD3ε.** The TCR complex is represented as a tight bundle as suggested by the recent model (23). (A) Prior to ligand binding, the cytoplasmic domains of ζ (red chains) could block the motif in CD3ε, which would become more accessible upon interaction with pMHC (11). (B) Alternatively, movement in CD3ε itself could lead to exposure of the proline motif (12).

rich region in thymocytes upon engagement with endogenous thymic ligands. Their detailed analysis of the CD3ε conformational change in a peptide-dependent manner lends support for a potential role for a conformational change.

The finding that both positively and negatively selecting ligands were capable of equivalently exposing the motif in CD3ε indicates that conformational change is not the discriminating factor in selection or for distinguishing agonist from antagonist. However, since peptides that do not function as ligands for the TCR did not induce a conformational change, some productive interaction is required to expose the proline motif. In this way, affinity itself does not appear to be a key factor in mediating conformational change. This is interesting when considering the previous structural studies of the A6 TCR interaction with altered peptide ligands (14). Interactions with both weak agonist and antagonists were very similar in structure to that of a strong agonist. These results were interpreted as providing evidence against a conformational change, but that assumed that altered peptide ligands would induce significantly different alterations in structure. Together, the structural and new biochemical data suggest that exposure of the proline motif in CD3ε alone is not sufficient for determining qualitative and/or quantitative differences in T cell signaling.

### Potential mechanisms for induction of CD3 conformational change

Although there are strong data supporting a conformational change in the CD3 cytoplasmic domains, the question remains how the change occurs upon ligand engagement. As discussed above, with the exception of the LC13 TCR crystal structure, most structural studies do not demonstrate a change in the membrane-proximal regions of the αβ heterodimer (9). However, a recent thermodynamic study, where change in heat capacity (a measure of binding energetics) correlates with conformational change or TCR flexibility, supports a role for conformational change (15). All but one of the analyzed peptides induced a conformational change in the TCR complex. Interestingly, the combination of half-life and conformational change and/or flexibility best predicted the level of T cell activation. Although the observed changes in heat capacity could be due strictly to changes in CDR3 loops, the recorded conformational change did have an impact on signaling outcome, based on IL-2 production by transgenic TCR T cells, suggesting that the change was somehow transmitted to the CD3 complex or ζ.

One mechanism to explain the alterations in CD3 is the piston model, which has been suggested for the bacterial aspartate receptor (16). A similar piston-like movement of the TCR or its rotation could make the cytoplasmic domains of the CD3 chains differentially accessible (12, 17, 18). For instance, structural rearrangement of the ζ chain could play a role in rendering the CD3ε chain and proline motif more accessible (11) (Fig. 1 A). Support for this comes from ζ-deficient mice reconstituted with ζ truncations. Truncation of ζ led to markedly increased phosphorylation of the CD3 chains after receptor stimulation (19). There is also some recent data to suggest that ζ may dissociate from the TCR complex or undergo conformational alterations after TCR ligation (20). In addition, according to this piston model, the conformational change in the A-B loop of the constant domain of TCRα observed for the LC13 TCR could potentially lead to exposure of the proline-rich region of CD3ε (9) (Fig. 1 B).

Although the TCR complex is often represented schematically as distinct units, biochemical studies have shown that the chains are associated and functionally coupled by their acidic and basic residue-containing transmembrane domains (8, 21). Biochemical studies support three-helix interactions between the transmembrane residues, where TCRα associates with CD3δε and the ζ homodimer, whereas TCRβ associates with CD3γε, supporting older cross-linking studies (8, 22). Recently, the ectodomain structure of the CD3εδ was published, thereby completing the structural analysis of the extracellular domains of the TCR complex (23). The authors of that study combined all of the available structural information and proposed a model in which the tight transmembrane association is reinforced by conserved ectodomain associations. They further hypothesized that the CD3 subunits sit under the constant domains of the TCR heterodimer loops because the majority of the CD3 dimers are inaccessible (Fig. 1). If the TCR complex is so tightly associated, it is clearly possible that small alterations that are induced upon ligand binding could be transmitted across the membrane to expose the proline-rich region in CD3ε directly or via a ζ conformational change (Fig. 1).

### Conclusion

The recent studies of the TCR complex suggest that ligand-induced conformational change does occur. However, the induction of this conformational change alone is not sufficient for T cell activation. Recent thermodynamic studies and the new study in this issue by Palmer and colleagues suggest that conformational change and other factors can account for signaling initiation and outcome (13, 15). Therefore, it remains to be seen how this conformational change contributes to T cell activation. Perhaps the conformational change is a prerequisite for ITAM phosphorylation, and some other unknown factor determines the persistence of phosphorylation and thus signaling potential. Future studies should concentrate on determining the mechanism for CD3ε conformational change and elucidating its biological relevance via mutagenesis of the proline-rich sequence.

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### REFERENCES

1. Cochran, J.R., D. Aivazian, T.O. Cameron, and L.J. Stern. 2001. Receptor clustering and transmembrane signaling in T cells. *Trends Biochem. Sci.* 26:304–310.

2. Irvine, D.J., M.A. Purbhoo, M. Krogsgaard, and M.M. Davis. 2002. Direct observation of ligand recognition by T cells. *Nature*. 419: 845–849.
3. Janeway, C.A., Jr. 1995. Ligands for the T-cell receptor: hard times for avidity models. *Immunol. Today*. 16:223–225.
4. Lanier, L.L., J.J. Ruitenberg, J.P. Allison, and A. Weiss. 1986. Distinct epitopes on the T cell antigen receptor of HPB-ALL tumor cells identified by monoclonal antibodies. *J. Immunol.* 137:2286–2292.
5. Yoon, S.T., U. Dianzani, K. Bottomly, and C.A. Janeway Jr. 1994. Both high and low avidity antibodies to the T cell receptor can have agonist or antagonist activity. *Immunity*. 1:563–569.
6. Hennecke, J., and D.C. Wiley. 2001. T cell receptor-MHC interactions up close. *Cell*. 104:1–4.
7. Reiser, J.B., C. Gregoire, C. Darnault, T. Mosser, A. Guimezanes, A.M. Schmitt-Verhulst, J.C. Fontecilla-Camps, G. Mazza, B. Malissen, and D. Housset. 2002. A T cell receptor CDR3beta loop undergoes conformational changes of unprecedented magnitude upon binding to a peptide/MHC class I complex. *Immunity*. 16:345–354.
8. Call, M.E., and K.W. Wucherpfennig. 2004. Molecular mechanisms for the assembly of the T cell receptor-CD3 complex. *Mol. Immunol.* 40:1295–1305.
9. Kjer-Nielsen, L., C.S. Clements, A.W. Purcell, A.G. Brooks, J.C. Whisstock, S.R. Burrows, J. McCluskey, and J. Rossjohn. 2003. A structural basis for the selection of dominant alphabeta T cell receptors in antiviral immunity. *Immunity*. 18:53–64.
10. Lin, J., and A. Weiss. 2001. T cell receptor signalling. *J. Cell Sci.* 114:243–244.
11. Aivazian, D., and L.J. Stern. 2000. Phosphorylation of T cell receptor zeta is regulated by a lipid dependent folding transition. *Nat. Struct. Biol.* 7:1023–1026.
12. Gil, D., W.W. Schamel, M. Montoya, F. Sanchez-Madrid, and B. Alarcón. 2002. Recruitment of Nck by CD3 epsilon reveals a ligand-induced conformational change essential for T cell receptor signaling and synapse formation. *Cell*. 109:901–912.
13. Gil, D., A.G. Schrum, B. Alarcón, and E. Palmer. 2005. TCR engagement by peptide–MHC ligands induces a conformational change in the CD3 complex of thymocytes. *J. Exp. Med.* 201:517–522.
14. Ding, Y.H., B.M. Baker, D.N. Garboczi, W.E. Biddison, and D.C. Wiley. 1999. Four A6-TCR/peptide/HLA-A2 structures that generate very different T cell signals are nearly identical. *Immunity*. 11:45–56.
15. Krogsgaard, M., N. Prado, E.J. Adams, X.L. He, D.C. Chow, D.B. Wilson, K.C. Garcia, and M.M. Davis. 2003. Evidence that structural rearrangements and/or flexibility during TCR binding can contribute to T cell activation. *Mol. Cell*. 12:1367–1378.
16. Ottemann, K.M., W. Xiao, Y.K. Shin, and D.E. Koshland Jr. 1999. A piston model for transmembrane signaling of the aspartate receptor. *Science*. 285:1751–1754.
17. Davis, M.M. 2002. A new trigger for T cells. *Cell*. 110:285–287.
18. Sun, Z.J., K.S. Kim, G. Wagner, and E.L. Reinherz. 2001. Mechanisms contributing to T cell receptor signaling and assembly revealed by the solution structure of an ectodomain fragment of the CD3 epsilon gamma heterodimer. *Cell*. 105:913–923.
19. van Oers, N.S., P.E. Love, E.W. Shores, and A. Weiss. 1998. Regulation of TCR signal transduction in murine thymocytes by multiple TCR zeta-chain signaling motifs. *J. Immunol.* 160:163–170.
20. La Gruta, N.L., H. Liu, S. Dilioglou, M. Rhodes, D.L. Wiest, and D.A. Vignali. 2004. Architectural changes in the TCR:CD3 complex induced by MHC:peptide ligation. *J. Immunol.* 172:3662–3669.
21. Tan, L., J. Turner, and A. Weiss. 1991. Regions of the T cell receptor alpha and beta chains that are responsible for interactions with CD3. *J. Exp. Med.* 173:1247–1256.
22. Call, M.E., J. Pyrdol, M. Wiedmann, and K.W. Wucherpfennig. 2002. The organizing principle in the formation of the T cell receptor-CD3 complex. *Cell*. 111:967–979.
23. Arnett, K.L., S.C. Harrison, and D.C. Wiley. 2004. Crystal structure of a human CD3-epsilon/delta dimer in complex with a UCHT1 single-chain antibody fragment. *Proc. Natl. Acad. Sci. USA*. 101:16268–16273.