Cite as: X. Li *et al.*, *Science*
10.1126/science.abe0918 (2020).

Pre-T cell receptors topologically sample self-ligands during thymocyte β -selection

Xiaolong Li^{1,2,3*}, Réka Mizsei^{1*}, Kemin Tan⁴, Robert J. Mallis^{1,5,6}, Jonathan S. Duke-Cohan^{1,2,3}, Aoi Akitsu^{1,2,3}, Paul W. Tetteh^{1,2}, Abhinav Dubey^{6,7}, Wonmuk Hwang^{8,9,10,11}, Gerhard Wagner⁶, Matthew J. Lang^{12,13}, Haribabu Arthanari^{6,7}, Jia-huai Wang^{1,2,6,7,14†}, Ellis L. Reinherz^{1,2,3†}

¹Laboratory of Immunobiology, Dana-Farber Cancer Institute, Boston, MA, USA. ²Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA. ³Department of Medicine, Harvard Medical School, Boston, MA, USA. ⁴Structural Biology Center, X-ray Science Division, Advanced Photon Source, Argonne National Laboratory, Lemont, IL, USA. ⁵Department of Dermatology, Harvard Medical School, Boston, MA, USA. ⁶Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA. ⁷Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA, USA. ⁸Department of Biomedical Engineering, Texas A&M University, College Station, TX, USA. ⁹Department of Materials Science & Engineering, Texas A&M University, College Station, TX, USA. ¹⁰Department of Physics & Astronomy, Texas A&M University, College Station, TX, USA. ¹¹School of Computational Sciences, Korea Institute for Advanced Study, Seoul, Republic of Korea. ¹²Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN, USA. ¹³Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA. ¹⁴Department of Pediatrics, Harvard Medical School, Boston, MA, USA.

*These authors contributed equally to this work.

†Corresponding author. Email: jwang@crystal.harvard.edu (J.W.); ellis_reinherz@dfci.harvard.edu (E.L.R.)

Self-discrimination, a critical but ill-defined molecular process programmed during thymocyte development, requires myriad pre-T cell receptors (preTCRs) and $\alpha\beta$ TCRs. Using X-ray crystallography, we show how a preTCR applies the concave β -sheet surface of its single variable domain (V β) to “horizontally” grab the protruding MHC α 2-helix. By contrast, $\alpha\beta$ TCRs purpose all six complementary-determining region (CDR) loops of their paired V α V β module to recognize peptides bound to MHC molecules (pMHCs) in “vertical” head-to-head binding. The preTCR topological fit ensures that CDR3 β reaches the peptide’s featured C-terminal segment for pMHC sampling, establishing the subsequent $\alpha\beta$ TCR canonical docking mode. “Horizontal” docking precludes germline CDR1 β – and CDR2 β –MHC binding to broaden β -chain repertoire diversification before $\alpha\beta$ TCR-mediated selection refinement. Thus, one subunit successively attunes the recognition logic of related multicomponent receptors.

Pluripotent progenitors seed the thymus and undergo massive expansion in a precisely orchestrated manner (1). Proliferation at the early CD4⁺CD8[−] double-negative (DN) thymocyte stages (DN1, DN2, and DN3a) is driven by stem cell factor, IL-7, and Notch (2, 3). Productive T cell receptor (TCR)- β -chain rearrangement and surface pre-T cell receptor (preTCR) expression begins a second stage of proliferation at DN3b with transitioning to DN4, immature single-positive (ISP) thymocytes, and early CD4⁺CD8⁺ double-positive (DP) thymocyte blasts. This second stage, referred to as β -selection, is required for $\alpha\beta$ T-lineage commitment, allelic exclusion to restrict β -chain clonotypes to one per cell, and development of thymocytes expressing $\alpha\beta$ TCRs (4, 5).

PreTCRs each comprise a unique somatically rearranged TCR β gene product disulfide-linked to an invariant transmembrane glycoprotein, pT α , which is thymocyte-specific. pT α lacks a ligand-binding variable domain, but possesses one membrane-proximal constant domain, which pairs with the β -chain constant domain (6). Although early studies suggested that preTCRs do not require ligands (7–10), instead signaling autonomously through site-specific self-oligomerization (11, 12), sequence analysis across mammalian species

has refuted this model (13, 14). Notch signaling, in part, mimics preTCR signaling to explain this paradox (15). More recently, it has been shown that pMHC molecules are preTCR ligands, promoting thymocyte development through interactions with V β (16–20). Following preTCR triggering, pT α transcription is shut off, Notch signaling is blunted, and TCR α transcription is initiated such that each pT $\alpha\beta$ heterodimer can be replaced by a TCR $\alpha\beta$ heterodimer (21). Consequently, the same β -chain is paired with an α -chain at the major DP thymocyte stage. Each TCR $\alpha\beta$ clonotype creates a cognate pMHC ligand specificity with its V α V β module. Desirable TCRs foster thymocyte survival, whereas autoreactive TCRs induce thymocyte apoptosis consequent to self-pMHC interaction (22).

Both the recognition logic for the obligate preTCR to $\alpha\beta$ TCR transition and the impact of preTCR-self-pMHC interaction on T lineage repertoire formation have been elusive. In this report, we present crystal structures of a preTCR β chain, the sole ligand-binding subunit of the preTCR, in complex with pMHC ligands, and accompanying functional data. The β chain used was N15 β and its ligand vesicular stomatitis virus octapeptide (VSV8) or variant bound to a truncated

version of a class I MHC (MHC-I) K^b molecule (termed K^b -t2) comprising the peptide-binding $\alpha 1\alpha 2$ domains. To facilitate N15 β -pMHC co-crystallization, we covalently linked N15 β with K^b -t2 (figs. S1 and S2). The representative structure of N15 β -VSV8- K^b -t2 revealed the topological fitting by which the concave C''C'CFG β -sheet face of the Ig-like N15V β domain grabs the apex of the convex $\alpha 2$ helical region of K^b -t2 (Fig. 1A). Closed- and open-book surface views (Fig. 1B) as well as detailed sample-related analyses (figs. S2 to S5 and tables S1 to S3) further delineated this fitting.

TCR $\alpha\beta$ heterodimers “vertically” bind a pMHC molecule in a canonical mode with V α and V β domains contacting N- and C-terminal segments of a peptide, respectively (23, 24). By contrast, in the structure of the N15 β -VSV8- K^b -t2 complex, N15 β bound to pMHC in a “horizontal” docking mode (Fig. 2A), yet with the CDR3 β loop contacting the C terminus of the peptide. In the vertical N15 $\alpha\beta$ -VSV8- K^b binding, all six CDR loops from both α and β subunits made contact in a head-on fashion (Fig. 2A). The TCR $\alpha\beta$ -pMHC interface is generally flat with an approximately 1240-2020-Å² buried surface area (BSA) (23). Had a single TCR β subunit assumed the same vertical binding to pMHC using its three CDR loops, the BSA would be only around 900-1000 Å², considerably less than the average protein-protein interaction (PPI) value of 1600 $\pm$ 400 Å² (25). Horizontal topological fitting offered a BSA of 1378 Å² (table S4). We then generated a composite view of the distribution of CDR loops and footprints of a preTCR β versus TCR $\alpha\beta$ binding on the same K^b molecule (Fig. 2B). Despite different approaches to pMHC (Fig. 2C), both preTCR and TCR $\alpha\beta$ were capable of associating with the same CD3 signaling subunits (fig. S6).

The inherent left-handed twist of the β -sheet platform of an MHC-I molecule caused the $\alpha 1$ and $\alpha 2$ helices on top of the sheet to form a convex point on the peptide-binding groove, most prominently for the $\alpha 2$ -helix (Fig. 1A) (24). The twisted C''C'CFG β sheet of the N15 β V-domain contoured as a concavity (Fig. 1A). Convex/concave topological fitting should be a feature of all preTCRs binding to pMHC. The binding-geometry positions CDR3 β to contact the pMHC, while orienting CDR1 β and CDR2 β loops away from the interface (Figs. 1A, 2C, and 3A and table S5). Only after TCR β pairs with TCR α at the DP thymocyte stage, does the resultant TCR $\alpha\beta$ heterodimer mediate potential CDR1-2 interactions with pMHC. The topological fitting skews N15 β binding toward the peptide C-terminal segment in the MHC groove (Fig. 3B), suggesting that TCR $\alpha\beta$ canonical docking polarity onto pMHC (23, 24) is preset at the β -selection stage.

The C-terminal half of the bound peptide was the most structurally featured segment of pMHC-I ligands as exemplified by overlaying four K^b bound peptides on VSV8- K^b -t2 structure (Fig. 3C). When loaded in an MHC-I molecule, a peptide's two termini are fixed in the groove by mainchain

hydrogen bonds to conserved MHC residues (26). Beginning at p4, the peptides bulged out and diverged in backbone conformations (Fig. 3C). Topological fit enabled preTCRs to recognize this featured C-terminal backbone display, complementing the limited number of exposed peptide side chains to shape the $\alpha\beta$ TCR repertoire as described below. A featured peptide conformation applied to MHC-II (fig. S7 and table S6) suggested analogous preTCR recognition. By contrast, certain non-classic MHCs lacked a regular peptide-binding groove (fig. S7).

The N15 β -VSV8- K^b -t2 interface had two distinct areas (Fig. 3A and 3D): (i) a large hydrophobic patch on the N15 β C''C'-face like the “palm” of a hand to contact the K^b -t2 $\alpha 2$ -helix and (ii) N15 β CC' and FG (CDR3 β) loops like the “thumb” and “fingers”, respectively. The thumb residue E42 on the N15 β CC' loop formed a specific electrostatic interaction with K^b -t2 residues R157 and K131. Finger residues W97, E100 and Q101 on the CDR3 β loop made extensive interactions with K^b -t2 at the $\alpha 2$ apex to facilitate CDR3 β loop sampling of the VSV8 C-terminal segment. These two contact areas are likely a general feature for preTCR-pMHC interaction.

The N15 β -VSV8- K^b -t2 interaction was somewhat degenerate (Fig. 3A and tables S3 and S5). Hydrophobic interactions in the palm area were promiscuous. Although N15 β -VSV8- K^b -t2 potentially had seven maximal interfacial hydrogen bonds, only a few were conserved (Fig. 3A). On average there should be 9 $\pm$ 5 hydrogen bonds in a PPI to afford sufficient specificity (25), where the number in N15 β -VSV8- K^b -t2 is rather low, implying plasticity in the fit. W97 on the CDR3 β loop tip had its large side chain distributing on either side of VSV8 (Fig. 3E and fig. S5), indicative of conformational variability. Transience may enable the CDR3 β loop to sample myriad self-peptides. To investigate sampling directly, the N15 β subunit was examined alone or with VSV8- K^b -t in solution via NMR relaxation dispersion (Rex). The N15 β W97 side-chain atom (W97Ne) showed a Rex effect only upon addition of VSV8- K^b -t (Fig. 3F and fig. S8), indicative of dynamic changes in the μ s-ms timescale and consistent with ligand-dependent chemical exchange.

To examine functional consequences of the observed preTCR interaction with pMHC, we seeded wild-type hematopoietic stem cells onto stroma expressing or lacking surface MHC (16) and followed thymocyte development (Fig. 4A). At DN3, both preTCR-independent (Notch-related) and preTCR-dependent cellular expansion occurs, whereas at DN4, signaling is dominated by the preTCR (27). Proliferation at the DN3 stage was observed at d9 independent of MHC expression, whereas progression to the DPsm stage only occurred robustly on the MHC⁺ stroma (Fig. 4B and fig. S9). By d13, progression to the DPsm stage was maintained on the MHC⁺ but strongly diminished on MHC⁻ stroma. DN4 cell numbers

were low across all experiments due to their short-lived but essential transitional nature (28).

Since the preTCR is expressed on a significant fraction of DN3 and DN4 thymocytes, we examined whether the presence or absence of pMHC recognition by the preTCR impacted functional preTCR β clonotype representation (Fig. 4C). The DN3 subset expansion was essentially independent of pMHC presence on stroma with similar clonotype representations. By contrast, at the DN4 stage, when cells also expressed functional β chains without TCR α chains, β -chain clonotype diversity was 10-fold higher in the presence of MHC (Fig. 4C). Clonotype expansion on the MHC⁺ stroma was due to inclusion of many distinct single-copy β chains absent from the MHC⁻ stromal cultures (table S7). DN3 and DN4 thymocyte β chains harvested from both types of stroma showed comparable CDR3 length and hydrophobicity (fig. S10).

Finally, we studied stromal cells selectively expressing VSV8-K^b (16) and stem cells from B6 Rag2^{-/-} mice (lacking endogenous TCR α and β chains) as retroviral transduction recipients. Either wild-type N15 β (wtN15 β) or one of five N15 β chains mutated at interface residues in the N15 β -VSV8-K^b-t2 structure (Fig. 4D) were transduced, FACS-purified, and cultured (fig. S11 to S13). All mutant constructs expressed surface preTCRs at comparable levels to wild type (fig. S14). On day 6 capturing the DN3a to DN3b transition, or day 10 highlighting DN4 and DP development, mutants 1, 2, and 4 significantly blocked developmental progression (Fig. 4E and fig. S15), confirming the crystallographically defined interaction site.

Like the $\alpha\beta$ TCR, the preTCR is a mechanosensor where bioforces load and enhance receptor-ligand bond lifetimes via the β subunit (16, 29). Linked preTCR structural transitions exhibit greater reversibility compared to those of TCR $\alpha\beta$, reflecting unpaired V β domain compliance. Facile preTCR signaling (30) fosters β -chain repertoire formation and T-lineage repertoire logic is programmed through alterations in pMHC-binding receptor structures. preTCRs exploit a topological fit that only permits CDR3 β to interact with pMHC, supporting self-pMHC reactivity and reduced ligand specificity to favor β -clonotype inclusiveness. Subsequently, beginning in DP thymocytes when TCR α replaces pT α , the same C''C'CFG β -sheet face of the V β domain combines with that of the V α domain to form the V α -V β module in the $\alpha\beta$ TCR, which prevents horizontal pMHC binding. Instead, a vertical geometry is mandated to foster greater specificity among pMHC interface interactions, testing all six CDRs through positive and negative selection. Laxity of pMHC binding permitted at the β -selection stage is eliminated, thereby augmenting self-versus-nonself discrimination. Successful passage through this gauntlet allows a diverse set of matured T-lineage cells to move into the periphery to discern

and confront in their vertical $\alpha\beta$ TCR mode abnormal cells altered by pathogens or cancerous transformations.

REFERENCES AND NOTES

1. K. Shortman, M. Egerton, G. J. Spangrude, R. Scollay, The generation and fate of thymocytes. *Semin. Immunol.* **2**, 3–12 (1990). [Medline](#)
2. U. Koch, E. Fiorini, R. Bénédict, V. Besseyrias, K. Schuster-Gossler, M. Pierres, N. R. Manley, A. Duarte, H. R. Macdonald, F. Radtke, Delta-like 4 is the essential, nonredundant ligand for Notch1 during thymic T cell lineage commitment. *J. Exp. Med.* **205**, 2515–2523 (2008). [doi:10.1084/jem.20080829](#) [Medline](#)
3. H. R. Rodewald, M. Ogawa, C. Haller, C. Waskow, J. P. DiSanto, Pro-thymocyte expansion by c-kit and the common cytokine receptor gamma chain is essential for repertoire formation. *Immunity* **6**, 265–272 (1997). [doi:10.1016/S1074-7613\(00\)80329-5](#) [Medline](#)
4. T. Kreslavsky, M. Gleimer, M. Miyazaki, Y. Choi, E. Gagnon, C. Murre, P. Sicsinski, H. von Boehmer, β -Selection-induced proliferation is required for $\alpha\beta$ T cell differentiation. *Immunity* **37**, 840–853 (2012). [doi:10.1016/j.immuni.2012.08.020](#) [Medline](#)
5. H. von Boehmer, The thymus in immunity and in malignancy. *Cancer Immunol. Res.* **2**, 592–597 (2014). [doi:10.1158/2326-6066.CIR-14-0070](#) [Medline](#)
6. C. Saint-Ruf, K. Ungewiss, M. Groettrup, L. Bruno, H. J. Fehling, H. von Boehmer, Analysis and expression of a cloned pre-T cell receptor gene. *Science* **266**, 1208–1212 (1994). [doi:10.1126/science.7973703](#) [Medline](#)
7. B. H. Koller, P. Marrack, J. W. Kappler, O. Smithies, Normal development of mice deficient in beta 2M, MHC class I proteins, and CD8⁺ T cells. *Science* **248**, 1227–1230 (1990). [doi:10.1126/science.2112266](#) [Medline](#)
8. M. J. Grusby, H. Auchincloss Jr., R. Lee, R. S. Johnson, J. P. Spencer, M. Zijlstra, R. Jaenisch, V. E. Papaioannou, L. H. Glimcher, Mice lacking major histocompatibility complex class I and class II molecules. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3913–3917 (1993). [doi:10.1073/pnas.90.9.3913](#) [Medline](#)
9. H. J. Fehling, A. Krotkova, C. Saint-Ruf, H. von Boehmer, Crucial role of the pre-T-cell receptor alpha gene in development of alpha beta but not gamma delta T cells. *Nature* **375**, 795–798 (1995). [doi:10.1038/375795a0](#) [Medline](#)
10. B. A. Irving, F. W. Alt, N. Killeen, Thymocyte development in the absence of pre-T cell receptor extracellular immunoglobulin domains. *Science* **280**, 905–908 (1998). [doi:10.1126/science.280.5365.905](#) [Medline](#)
11. S. S. Pang, R. Berry, Z. Chen, L. Kjer-Nielsen, M. A. Perugini, G. F. King, C. Wang, S. H. Chew, N. L. La Gruta, N. K. Williams, T. Beddoe, T. Tiganis, N. P. Cowieson, D. I. Godfrey, A. W. Purcell, M. C. Wilce, J. McCluskey, J. Rossjohn, The structural basis for autonomous dimerization of the pre-T-cell antigen receptor. *Nature* **467**, 844–848 (2010). [doi:10.1038/nature09448](#) [Medline](#)
12. S. Yamasaki, E. Ishikawa, M. Sakuma, K. Ogata, K. Sakata-Sogawa, M. Hiroshima, D. L. Wiest, M. Tokunaga, T. Saito, Mechanistic basis of pre-T cell receptor-mediated autonomous signaling critical for thymocyte development. *Nat. Immunol.* **7**, 67–75 (2006). [doi:10.1038/ni1290](#) [Medline](#)
13. P. Smeltz, C. Marchal, R. Renard, L. Sinzelle, N. Pollet, D. Dunon, T. Jaffredo, J.-Y. Sire, J. S. Fella, Identification of the pre-T-cell receptor alpha chain in nonmammalian vertebrates challenges the structure-function of the molecule. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 19991–19996 (2010). [doi:10.1073/pnas.1010166107](#) [Medline](#)
14. B. Zhou, Q. Chen, R. J. Mallis, H. Zhang, J. H. Liu, E. L. Reinherz, J. H. Wang, A conserved hydrophobic patch on V β domains revealed by TCR β chain crystal structures: Implications for pre-TCR dimerization. *Front. Immunol.* **2**, 5 (2011). [doi:10.3389/fimmu.2011.00005](#) [Medline](#)
15. A. M. Michie, A. C. Chan, M. Ciofani, M. Carleton, J. M. Lefebvre, Y. He, D. M. Allman, D. L. Wiest, J. C. Zúñiga-Pflücker, D. J. Izon, Constitutive Notch signalling promotes CD4 CD8 thymocyte differentiation in the absence of the pre-TCR complex, by mimicking pre-TCR signals. *Int. Immunol.* **19**, 1421–1430 (2007). [doi:10.1093/intimm/dxm113](#) [Medline](#)
16. D. K. Das, R. J. Mallis, J. S. Duke-Cohan, R. E. Hussey, P. W. Tetteh, M. Hilton, G. Wagner, M. J. Lang, E. L. Reinherz, Pre-T cell receptors (Pre-TCRs) leverage V β complementarity determining regions (CDRs) and hydrophobic patch in mechanosensing thymic self-ligands. *J. Biol. Chem.* **291**, 25292–25305 (2016). [doi:10.1074/jbc.M116.752865](#) [Medline](#)
17. R. J. Mallis, H. Arthanari, M. J. Lang, E. L. Reinherz, G. Wagner, NMR-directed

- design of pre-TCR β and pMHC molecules implies a distinct geometry for pre-TCR relative to $\alpha\beta$ TCR recognition of pMHC. *J. Biol. Chem.* **293**, 754–766 (2018). [doi:10.1074/jbc.M117.813493](https://doi.org/10.1074/jbc.M117.813493) [Medline](#)
18. R. J. Mallis, K. Bai, H. Arthanari, R. E. Hussey, M. Handley, Z. Li, L. Chingozha, J. S. Duke-Cohan, H. Lu, J. H. Wang, C. Zhu, G. Wagner, E. L. Reinherz, Pre-TCR ligand binding impacts thymocyte development before $\alpha\beta$ TCR expression. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 8373–8378 (2015). [doi:10.1073/pnas.1504971112](https://doi.org/10.1073/pnas.1504971112) [Medline](#)
 19. R. J. Mallis, K. N. Brazin, J. S. Duke-Cohan, W. Hwang, J. H. Wang, G. Wagner, H. Arthanari, M. J. Lang, E. L. Reinherz, NMR: An essential structural tool for integrative studies of T cell development, pMHC ligand recognition and TCR mechanobiology. *J. Biomol. NMR* **73**, 319–332 (2019). [doi:10.1007/s10858-019-00234-8](https://doi.org/10.1007/s10858-019-00234-8) [Medline](#)
 20. X. Yang, R. A. Mariuzza, Pre-T-cell receptor binds MHC: Implications for thymocyte signaling and selection. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 8166–8167 (2015). [doi:10.1073/pnas.1510127112](https://doi.org/10.1073/pnas.1510127112) [Medline](#)
 21. C. López-Rodríguez, J. Aramburu, R. Berga-Bolaños, Transcription factors and target genes of pre-TCR signaling. *Cell. Mol. Life Sci.* **72**, 2305–2321 (2015). [doi:10.1007/s00018-015-1864-8](https://doi.org/10.1007/s00018-015-1864-8) [Medline](#)
 22. L. Klein, B. Kyewski, P. M. Allen, K. A. Hogquist, Positive and negative selection of the T cell repertoire: What thymocytes see (and don't see). *Nat. Rev. Immunol.* **14**, 377–391 (2014). [doi:10.1038/nri3667](https://doi.org/10.1038/nri3667) [Medline](#)
 23. M. G. Rudolph, R. L. Stanfield, I. A. Wilson, How TCRs bind MHCs, peptides, and coreceptors. *Annu. Rev. Immunol.* **24**, 419–466 (2006). [doi:10.1146/annurev.immunol.23.021704.115658](https://doi.org/10.1146/annurev.immunol.23.021704.115658) [Medline](#)
 24. J. H. Wang, E. L. Reinherz, The structural basis of $\alpha\beta$ T-lineage immune recognition: TCR docking topologies, mechanotransduction, and co-receptor function. *Immunol. Rev.* **250**, 102–119 (2012). [doi:10.1111/j.1600-065X.2012.01161.x](https://doi.org/10.1111/j.1600-065X.2012.01161.x) [Medline](#)
 25. L. Lo Conte, C. Chothia, J. Janin, The atomic structure of protein-protein recognition sites. *J. Mol. Biol.* **285**, 2177–2198 (1999). [doi:10.1006/jmbi.1998.2439](https://doi.org/10.1006/jmbi.1998.2439) [Medline](#)
 26. C. Zhang, A. Anderson, C. DeLisi, Structural principles that govern the peptide-binding motifs of class I MHC molecules. *J. Mol. Biol.* **281**, 929–947 (1998). [doi:10.1006/jmbi.1998.1982](https://doi.org/10.1006/jmbi.1998.1982) [Medline](#)
 27. H. Hosokawa, E. V. Rothenberg, Cytokines, transcription factors, and the initiation of T-cell development. *Cold Spring Harb. Perspect. Biol.* **10**, a028621 (2018). [doi:10.1101/cshperspect.a028621](https://doi.org/10.1101/cshperspect.a028621) [Medline](#)
 28. F. Vasseur, A. Le Campion, C. Pénit, Scheduled kinetics of cell proliferation and phenotypic changes during immature thymocyte generation. *Eur. J. Immunol.* **31**, 3038–3047 (2001). [doi:10.1002/1521-4141\(2001010\)31:10<3038::AID-IMMU3038>3.0.CO;2-3](https://doi.org/10.1002/1521-4141(2001010)31:10<3038::AID-IMMU3038>3.0.CO;2-3) [Medline](#)
 29. D. K. Das, Y. Feng, R. J. Mallis, X. Li, D. B. Keskin, R. E. Hussey, S. K. Brady, J. H. Wang, G. Wagner, E. L. Reinherz, M. J. Lang, Force-dependent transition in the T-cell receptor β -subunit allosterically regulates peptide discrimination and pMHC bond lifetime. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 1517–1522 (2015). [doi:10.1073/pnas.1424829112](https://doi.org/10.1073/pnas.1424829112) [Medline](#)
 30. Y. Feng, E. L. Reinherz, M. J. Lang, $\alpha\beta$ T cell receptor mechanosensing forces out serial engagement. *Trends Immunol.* **39**, 596–609 (2018). [doi:10.1016/j.it.2018.05.005](https://doi.org/10.1016/j.it.2018.05.005) [Medline](#)
 31. M. K. Teng, A. Smolyar, A. G. Tse, J. H. Liu, J. Liu, R. E. Hussey, S. G. Nathenson, H. C. Chang, E. L. Reinherz, J. H. Wang, Identification of a common docking topology with substantial variation among different TCR-peptide-MHC complexes. *Curr. Biol.* **8**, 409–414 (1998). [doi:10.1016/S0960-9822\(98\)70160-5](https://doi.org/10.1016/S0960-9822(98)70160-5) [Medline](#)
 32. W. Minor, M. Cymborowski, Z. Otwinowski, M. Chruszcz, HKL-3000: The integration of data reduction and structure solution—from diffraction images to an initial model in minutes. *Acta Crystallogr. D Biol. Crystallogr.* **62**, 859–866 (2006). [doi:10.1107/S0907444906019949](https://doi.org/10.1107/S0907444906019949) [Medline](#)
 33. M. D. Winn, C. C. Ballard, K. D. Cowtan, E. J. Dodson, P. Emsley, P. R. Evans, R. M. Keegan, E. B. Krissinel, A. G. Leslie, A. McCoy, S. J. McNicholas, G. N. Murshudov, N. S. Pannu, E. A. Potterton, H. R. Powell, R. J. Read, A. Vagin, K. S. Wilson, Overview of the CCP4 suite and current developments. *Acta Crystallogr. D Biol. Crystallogr.* **67**, 235–242 (2011). [doi:10.1107/S0907444910045749](https://doi.org/10.1107/S0907444910045749) [Medline](#)
 34. A. J. McCoy, R. W. Grosse-Kunstleve, P. D. Adams, M. D. Winn, L. C. Storoni, R. J. Read, Phaser crystallographic software. *J. Appl. Crystallogr.* **40**, 658–674 (2007). [doi:10.1107/S0021889807021206](https://doi.org/10.1107/S0021889807021206) [Medline](#)
 35. P. D. Adams, P. V. Afonine, G. Bunkóczi, V. B. Chen, I. W. Davis, N. Echols, J. J. Headd, L. W. Hung, G. J. Kapral, R. W. Grosse-Kunstleve, A. J. McCoy, N. W. Moriarty, R. Oeffner, R. J. Read, D. C. Richardson, J. S. Richardson, T. C. Terwilliger, P. H. Zwart, PHENIX: A comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 213–221 (2010). [doi:10.1107/S0907444909052925](https://doi.org/10.1107/S0907444909052925) [Medline](#)
 36. P. Emsley, B. Lohkamp, W. G. Scott, K. Cowtan, Features and development of Coot. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 486–501 (2010). [doi:10.1107/S0907444910007493](https://doi.org/10.1107/S0907444910007493) [Medline](#)
 37. P. V. Afonine, R. W. Grosse-Kunstleve, N. Echols, J. J. Headd, N. W. Moriarty, M. Mustyakimov, T. C. Terwilliger, A. Urzhumtsev, P. H. Zwart, P. D. Adams, Towards automated crystallographic structure refinement with phenix.refine. *Acta Crystallogr. D Biol. Crystallogr.* **68**, 352–367 (2012). [doi:10.1107/S0907444912001308](https://doi.org/10.1107/S0907444912001308) [Medline](#)
 38. M. C. Lawrence, P. M. Colman, Shape complementarity at protein/protein interfaces. *J. Mol. Biol.* **234**, 946–950 (1993). [doi:10.1006/jmbi.1993.1648](https://doi.org/10.1006/jmbi.1993.1648) [Medline](#)
 39. F. Delaglio, S. Grzesiek, G. W. Vuister, G. Zhu, J. Pfeifer, A. Bax, NMRPipe: A multidimensional spectral processing system based on UNIX pipes. *J. Biomol. NMR* **6**, 277–293 (1995). [doi:10.1007/BF00197809](https://doi.org/10.1007/BF00197809) [Medline](#)
 40. S. G. Hyberts, A. G. Milbradt, A. B. Wagner, H. Arthanari, G. Wagner, Application of iterative soft thresholding for fast reconstruction of NMR data non-uniformly sampled with multidimensional Poisson Gap scheduling. *J. Biomol. NMR* **52**, 315–327 (2012). [doi:10.1007/s10858-012-9611-z](https://doi.org/10.1007/s10858-012-9611-z) [Medline](#)
 41. R. Keller, *The Computer Aided Resonance Assignment* (Cantina, 2004).
 42. J. P. Loria, M. Rance, A. G. Palmer 3rd, A TROSY CPMG sequence for characterizing chemical exchange in large proteins. *J. Biomol. NMR* **15**, 151–155 (1999). [doi:10.1023/A:1008355631073](https://doi.org/10.1023/A:1008355631073) [Medline](#)
 43. J. P. Loria, M. Rance, A. G. Palmer, A relaxation-compensated Carr-Purcell-Meiboom-Gill sequence for characterizing chemical exchange by NMR spectroscopy. *J. Am. Chem. Soc.* **121**, 2331–2332 (1999). [doi:10.1021/ja983961a](https://doi.org/10.1021/ja983961a)
 44. M. Bieri, E. J. d'Auvergne, P. R. Gooley, relaxGUI: A new software for fast and simple NMR relaxation data analysis and calculation of ps-ns and μ s motion of proteins. *J. Biomol. NMR* **50**, 147–155 (2011). [doi:10.1007/s10858-011-9509-1](https://doi.org/10.1007/s10858-011-9509-1) [Medline](#)
 45. S. Morin, T. E. Linnet, M. Lescanne, P. Schanda, G. S. Thompson, M. Tollinger, K. Teilum, S. Gagné, D. Marion, C. Griesinger, M. Blackledge, E. J. d'Auvergne, relax: The analysis of biomolecular kinetics and thermodynamics using NMR relaxation dispersion data. *Bioinformatics* **30**, 2219–2220 (2014). [doi:10.1093/bioinformatics/btu166](https://doi.org/10.1093/bioinformatics/btu166) [Medline](#)
 46. Z. Luz, S. Meiboom, Nuclear magnetic resonance study of the protolysis of trimethylammonium ion in aqueous solution—order of the reaction with respect to solvent. *J. Chem. Phys.* **39**, 366–370 (1963). [doi:10.1063/1.1734254](https://doi.org/10.1063/1.1734254)
 47. D. Long, G. Bouvignies, L. E. Kay, Measuring hydrogen exchange rates in invisible protein excited states. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 8820–8825 (2014). [doi:10.1073/pnas.1405011111](https://doi.org/10.1073/pnas.1405011111) [Medline](#)
 48. I. Z. Mamedov, O. V. Britanova, I. V. Zvyagin, M. A. Turchaninova, D. A. Bolotin, E. V. Putintseva, Y. B. Lebedev, D. M. Chudakov, Preparing unbiased T-cell receptor and antibody cDNA libraries for the deep next generation sequencing profiling. *Front. Immunol.* **4**, 456 (2013). [doi:10.3389/fimmu.2013.00456](https://doi.org/10.3389/fimmu.2013.00456) [Medline](#)
 49. D. A. Bolotin, S. Poslavsky, I. Mitrophanov, M. Shugay, I. Z. Mamedov, E. V. Putintseva, D. M. Chudakov, MiXCR: Software for comprehensive adaptive immunity profiling. *Nat. Methods* **12**, 380–381 (2015). [doi:10.1038/nmeth.3364](https://doi.org/10.1038/nmeth.3364) [Medline](#)
 50. M. Shugay, D. V. Bagaev, M. A. Turchaninova, D. A. Bolotin, O. V. Britanova, E. V. Putintseva, M. V. Pogorelyy, V. I. Nazarov, I. V. Zvyagin, V. I. Kirgizova, K. I. Kirgizov, E. V. Skorobogatova, D. M. Chudakov, VDJtools: Unifying Post-analysis of T Cell Receptor Repertoires. *PLOS Comput. Biol.* **11**, e1004503 (2015). [doi:10.1371/journal.pcbi.1004503](https://doi.org/10.1371/journal.pcbi.1004503) [Medline](#)
 51. J. Kyte, R. F. Doolittle, A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**, 105–132 (1982). [doi:10.1016/0022-2836\(82\)90515-Q](https://doi.org/10.1016/0022-2836(82)90515-Q) [Medline](#)
 52. S. Fuchs, “GRAVY calculator” (2020); <http://www.gravy-calculator.de/>.

ACKNOWLEDGMENTS

We thank E. Dodson for helpful discussion in crystallography. **Funding:** This work was supported by NIH grants P01AI143565, AI136960, AI136301, GM047467, AI0037581 and EB002026, a Rosztoczy Foundation Scholarship and a Claudia Adams Barr award. The use of SBC 19-ID at Argonne National Laboratory was supported by DOE Contract No. DE-AC02-06CH11357. **Author contributions:** E.L.R. and J.H.W. conceived, organized, and supervised the project. E.L.R., J.H.W. and X.L. prepared the manuscript, with input from all authors. R.M. and R.J.M. designed, cloned, refolded, and purified protein samples. X.L. produced crystals. X.L., K.T., and J.H.W. collected and processed diffraction data and determined and refined the structure models. X.L. deposited the structures. R.J.M., A.D., G.W., and H.A. performed NMR experiments. J.S.D-C. performed thymic repertoire analysis. A.A. performed cell and FACS experiments and animal breeding. J.H.W., X.L., K.T., and E.L.R. analyzed X-ray crystal structures. R.J.M., A.D., G.W., and H.A. analyzed NMR data. J.S.D-C., A.A., and E.L.R. analyzed functionally experimental data. **Competing interests:** G.W. is a co-founder and has equity in the following companies: Enanta Pharmaceuticals, PIC therapeutics, Cellmig biolabs, Skinap therapeutics, Virtual Discovery, Olaris therapeutics. However, none of these companies are related to the topic of this manuscript. The remaining authors declare no competing interests. **Data and materials availability:** All structural data have been deposited in the Protein Data Bank (<https://www.rcsb.org>), with PDB codes: 6WL2, 6WL3, 6WL4, 7JI2. All other data associated with this study are in the main text or supplementary materials.

SUPPLEMENTARY MATERIALS

science.sciencemag.org/cgi/content/full/science.abe0918/DC1

Materials and Methods

Figs. S1 to S16

Tables S1 to S8

References (32–52)

MDAR Reproducibility Checklist

30 July 2020; accepted 3 December 2020

Published online 17 December 2020

10.1126/science.abe0918

Fig. 1. The structure of N15β-VSV8-K^b-t2. (A) Ribbon drawing of one representative structure of N15β-VSV8-K^b-t2. The twisted concave C''C'CFG β sheet of N15β V-domain topologically fits the protruding α2-helix of K^b-t2 with N15β CDR3 loop reaching the C-terminal VSV8 segment. Note that CDR1 and CDR2 point away from pMHC. (B) Surface representation of the "closed-book" view (top panel) and "open-book" view (bottom panel) of N15β-VSV8-K^b-t2.

Fig. 2. Distinct pMHC-binding mode of preTCR versus TCR $\alpha\beta$. (A) Side-by-side ribbon drawings of the “horizontal” N15preTCR–VSV8-K^b complex model (left) and “vertical” N15TCR $\alpha\beta$ –VSV8-K^b complex structure (right) (31). The former was constructed by overlaying structures of human preTCR (PDB: 3OF6) and mouse VSV8-K^b (PDB: 1KPU) onto the current structure of N15 β –VSV8-K^b-t2. (B) Composite view of the footprints of a TCR $\alpha\beta$ (PDB: 2CKB) and the preTCR on the same K^b (gray surface and green ribbon). The β -chain footprints of TCR $\alpha\beta$ and preTCR are delineated by magenta and blue dashed lines, respectively. All CDR loops are displayed. The VSV8 is in red ribbon representation with its exposed residues V4 and Q6 shown. (C) Simplified illustration of pMHC binding of preTCR versus TCR $\alpha\beta$ with atomistic model comparison in Fig. S6. Only CDR3 is labeled.

Fig. 3. The interactions of N15 β with VSV8-K^b-t2. (A) The interaction network of N15 β with K^b-t2 and VSV8 (4-Å cutoff). Black dashed lines, black solid lines, and green lines denote those interactions observed in 1-3, 4-6, or 7-9 complexes in three crystals, respectively. N15 β residues in orange and blue are located in two distinct K^b-t2-contacting regions, respectively (see Fig. 3D). (B) N15 β binding to the VSV8 peptide. Only the C-terminal part of VSV8, particularly V4 and Q6 are involved in the N15 β -contact. Dashed lines represent hydrogen bonds. (C) Two K^b-binding octapeptides (OVA and OVA variant Q4H7) and nonapeptides (SEV9 and α -glucosidase) are superimposed on VSV8-K^b-t2. The backbone of N-terminal p1-p3 peptide residues is held in the binding-groove by hydrogen-bonds to conserved MHC residues, whereas their protruding C-terminal residues diverge in backbone conformation. (D) Two distinct VSV8-K^b-t2-binding areas on N15 β are the “palm” in orange and the “thumb and fingers” in blue. Their interacting residues on K^b-t2 are colored in cyan. (E) Conformational variability of W97 on the N15 β CDR3 β loop. Numbers in parentheses represent the frequency of each contact among the nine complexes. Note the W97 side chain can position on either side of the VSV8 peptide. (F) ¹⁵N relaxation dispersion profiles of W97 show changes consistent with chemical exchange on ligand binding with addition of VSV8-K^b-t. Blue circles and green points represent measured transverse relaxation (R_2) at 700 and 600 MHz, respectively, at a given Carr-Purcell-Meiboom-Gill pulse frequency (ν_{CPMG}).

Fig. 4. Impact of preTCR-pMHC interactions on β -chain repertoire expansion. (A) Schematic for thymocyte development. B6 hematopoietic stem cells (HSCs) (light blue and magenta) were cultured on OP9-DL4 cells (light brown) expressing or lacking MHC. For simplicity, the numerically minor immature single-positive thymocytes prior to DP blast (DPbl) are omitted with subpopulations expressing preTCRs starting at DN3 and persisting through DN4 denoted by asterisks. (B) Thymocyte cell number per stage (DN3 to DPsmall (DPsm)) at d9 and d13 after HSC seeding (2000 cells) on MHC⁺ or MHC⁻ OP9-DL4 stromal cells. Each symbol (●, ■, ▲, ◆) represents an independent experiment (n=4). *: $P < 0.05$; **: $P < 0.02$; ***, $P < 0.01$ using two-tailed Student's *t* test. (C) preTCR β -chain unique clonotype representation in 10^4 DN3 and DN4 cells after 9 days of growth on MHC⁺ or MHC⁻ stromal cells. For each condition, the box bounds the 25th and 75th percentile with internal 50th percentile (solid line) and mean (dotted line). Whiskers with bars define upper and lower values for each condition (n=5). For DN4, $P < 0.05$ on comparison of MHC⁺ and MHC⁻ conditions. (D) Key interface contacts in the N15 β -VSV8-K^b-t2 structure used for mutagenesis studies in (E). (E) upper row highlights DN3a-DN3b transition while lower row shows later DN4 and DP changes (n=3). *: $P < 0.05$; **: $P < 0.01$ using two-tailed Student's *t* test. DN3a, DN3b, and DN4 percentages were derived by gating on the CD4⁺CD8⁺CD44⁺ population (fig. S12). Shading indicates interface mutants that significantly inhibit thymocyte developmental progression.