IMMUNOLOGY

Engineered retroviruses map ligand–receptor interactions

Engineered viral entry combined with single-cell sequencing technology makes it possible to identify specific ligand–receptor interactions in a high-throughput manner.

Yuqian Wang, Zhe Wang and Guideng Li

The maintenance of body homeostasis is highly dependent on on the recognition and clearance of 'non-self' substances by the immune system, mediated by an immense number of finely tuned ligand–receptor interactions. A huge diversity of antigens can be recognized by antigen receptors on T and B lymphocytes (namely, B cell receptors (BCRs) and T cell receptors (TCRs)). V(D)J recombination results in the diversity of the BCR and TCR repertoires, which recognize membrane-bound proteins and peptide–major histocompatibility complex (pMHC) assemblies, respectively. It is estimated that the TCR repertoire of an individual contains about 10^{12} unique clones. A single TCR can interact with millions of different antigenic peptides and a single peptide may be recognized by several TCRs, further increasing the complexity of TCR–pMHC interactions. The ability to rapidly resolve ligand–receptor interactions remains a bottleneck in the field of infectious disease, autoimmunity and cancer immunology. In this issue of *Nature Methods*, Dobson et al.[1] describe an elegant approach, termed receptor–antigen pairing by targeted retroviruses (RAPTR), to decode ligand–receptor interactions (Fig. 1).

In recent decades, many methods have been established to identify antigen-specific TCRs and BCRs[2,3]. pMHC tetramers are an antigen-directed approach that is widely used to label and identify antigen-specific T cells. Recently developed DNA-barcoded pMHC tetramers allow high-throughput screening[4] in which, theoretically, up to 10^{10} antigens can be screened simultaneously. However, the use of pMHC tetramers is limited by the low throughput of peptides synthesis and the inconvenience of pMHC tetramer assembly. TCR-directed approaches based on yeast or mammalian cell display systems — such as signaling and antigen-presenting bifunctional receptors (SABR)[5], trogocytosis[6], T-scan[7], granzyme B-based target cell labeling[8] and TCR–MCR[9] — have also been developed to deorphanize TCRs. For example, the trogocytosis-based approach uses the transfer of membrane proteins to label the cognate antigen-presenting cells for a given TCR, and has been used to successfully deorphanize a neoantigen-specific TCR from a patient with melanoma[6]. However, TCR-antigen screening methods remain limited by time-consuming processes that are caused by multiple rounds of screening, inefficiencies associated with peptide synthesis, poor sensitivity for low-affinity antigens or unavailability for 'library-on-library' screening. Fluorescently labeled and/or oligonucleotide-conjugated recombinant proteins are commonly used to identify antigen-specific B cells[3]; however, these approaches also encounter challenges involved with large-scale protein synthesis, similarly to the pMHC tetramer method.

An ideal method to decode ligand–receptor interaction would directly obtain both ligand and receptor information in a simple and high-throughput manner. Dobson et al.[1] have developed a robust method known as RAPTR, which uses an engineered retrovirus to explore specific interactions and simultaneously acquire both ligand and receptor information. They have innovatively exploited pseudotyped lentiviruses with mutant VSV-G (VSV-G mut (K37Q, R354Q) with no binding ability as a fusogen to display a given membrane protein on its surface[10]. The specific entry of the virus into target cells is enabled by the interaction of the membrane protein displayed on the viral surface with its cognate receptor on target cells. Dobson et al.[1] demonstrate that ligand-displaying VSV-G mut pseudotyping virus can infect target Jurkat cells via cytokine receptors, costimulatory receptors, BCRs and TCRs with high selectivity and specificity. To ensure that the viral particle presents only a given ligand during library-on-library screening, the authors modified the lentiviral vector genome and packaging process to enable each packaging cell to produce viruses that match viral-surface phenotype and genotype, therefore allowing scalable construction of viral libraries. They applied RAPTR to profile BCRs using a defined antigen library containing 43 viral-surface antigens, and successfully enriched SARS-CoV spike proteins both for a naive and mature BCR that are cross-reactive with the RBD domain of SARS-CoV and SARS-CoV-2. Thus, this work shows the potential of RAPTR to deorphanize receptors and assess BCR cross-reactivity. RAPTR was further used for TCR-antigen screening and successfully enriched cytomegalovirus-derived and influenza A-derived peptides for their respective cognate TCRs. Combining the method with single-cell sequencing, the authors[1] performed a library-on-library screen on TCR-expressing Jurkat T cells that were pre-enriched by tetramer from a pool of T cells containing over 450,000 TCRs, and successfully identified GL9-specific (influenza A-derived peptide) and GLC-specific (Epstein–Barr virus-derived peptide) TCR clones.

RAPTR is an integrated 'all-in-one' method that combines ligand display, cargo delivery and interaction readout for massively parallel decoding of ligand–receptor interactions. Compared with current TCR-antigen screening methods, RAPTR is a convenient method that can be adapted by a biological laboratory with basic facilities. Taking advantage of host mammalian cells, RAPTR overcomes the limitations of yeast- or phage-display methods and presents a wide range of membrane proteins with proper folding and modifications, ensuring proper identification of genuine ligand–receptor interactions. In addition, the pseudotyped lentiviruses contain more

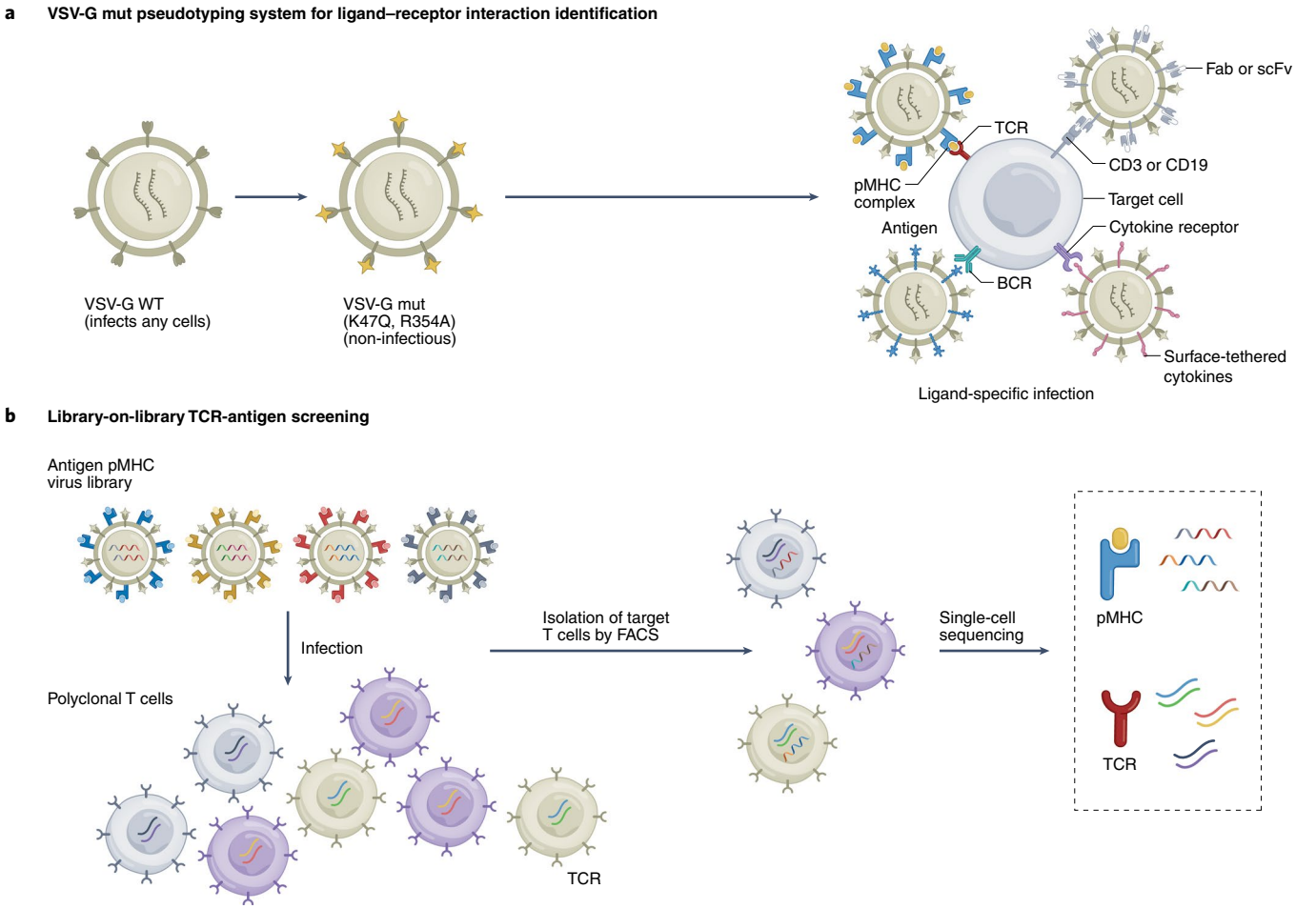

Fig. 1 | Schematic overview of VSV-G mut (K47Q, R354A) pseudotyping system for identifying specific ligand–receptor interactions. a, The VSV-G-mut pseudotyped lentivirus is used to display a variety of membrane proteins on its surface. Specific viral entry is mediated by the virus-displayed protein interacting with its receptors on target cells, including membrane signaling and cytokine receptors, immune cell membrane markers and membrane-anchored proteins. WT, wild type; Fab, antibody-binding fragment; scFv, single-chain variable fragment. **b**, T cells of interest are infected with a pool of VSV-G-mut lentiviruses that display different pMHC complexes. The transduced cells are isolated and then subjected to single-cell sequencing to directly uncover the sequence information of paired TCRs and antigens. FACS, fluorescence-activated cell sorting.

molecules for a given T cell ligand on their surface than do pMHC tetramers; thus, RAPTR may be a more sensitive method to qualify antigen-specific T cells (particularly those T cells bearing low-affinity TCRs). Given the high efficiency of RAPTR in screening TCRs and BCRs using cell lines, RAPTR could easily be applied to the profiling and isolation of rare antigen-specific primary T or B cells. Additionally, in combination with single-cell sequencing technology, RAPTR can be optimized to simultaneously obtain transcriptome information and molecular phenotypes of antigen-specific T or B cells at single-cell resolution. However, a major limitation of the RAPTR approach is that preparation of the large-scale virus-displaying library for interactome studies is still time-consuming and labor-intensive. A scalable and fully automated platform would be needed to address this challenge encountered during library preparation.

RAPTR is a powerful tool to systematically decode protein ligand–receptor interactions in individuals under different physiological and pathological conditions, which will greatly improve our understanding of the mechanisms of immune recognition and evasion, as well as of all aspects of cell–cell communications beyond the immune system. The interactome-mediated cell transduction characteristic of RAPTR also opens new avenues for cell type-specific gene delivery and gene editing. ❐

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Competing interests
The authors declare no competing interests.

NEUROSCIENCE

Tracking together: estimating social poses

Two new toolkits that leverage deep-learning approaches can track the positions of multiple animals and estimate poses in different experimental paradigms.

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The study of social behavior has a reputation of being an imprecise science. Despite the essential importance of complex and multifarious interactions between individuals for survival and reproduction, most experiments in the field rely on analyzing simple measures, such as the amount of time an animal spends on one side of a box, or human-defined behaviors that rely on an intense amount of manual annotation. The former approach is unable to capture the full richness of the underlying behavior, and the latter is subject to the inevitable biases that result from human observations.

Limiting progress toward a more principled and automated approach to social behavior has been the medium of capture itself — videography. Although videos contain a wealth of behavioral information, they also are typically difficult to parse, especially in experiments with complicated visual backgrounds or where several animals are interacting with and occluding each other. Over the past few years, advances in machine learning have improved our ability to track the postures of individual animals to new levels of accuracy and accessibility[1–4]. However, tracking the poses of multiple animals has remained a substantial challenge. One reason is that the points detected on the body parts of the animals swap or disappear during close interaction, leading to uninterpretable analyses and frustrated scientists. Studies published in this issue of *Nature Methods*[5,6] leverage deep-learning approaches to detect and maintain the tracked postures of several animals in a video, increasing our ability to gain insights into the social interactions of these animals and — eventually — to understand the physiological processes that underlie them.

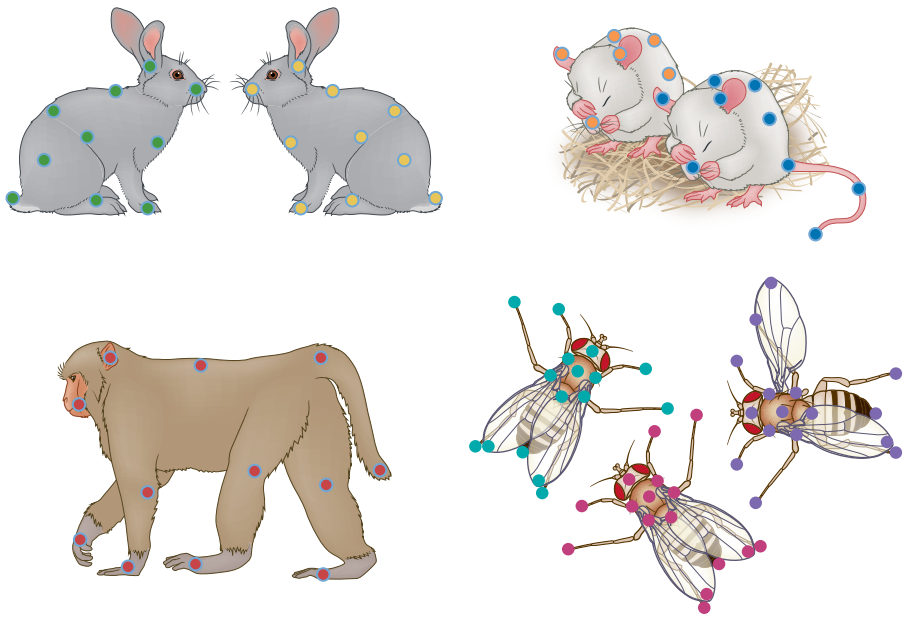

Fig. 1 | Estimating postures of single and multiple animals. Using deep-learning approaches, maDLC and SLEAP offer tools that are species-agnostic to track animal posture in different experimental paradigms.

Prior to these studies, tools for tracking multiple animals have largely focused on tracking the centroid and orientation of the animals. These approaches used computer vision and deep-learning tools to maintain the animals' identities while they interacted[7–10], allowing us to gain insights into the trajectories of these animals in a social context. However, these methods only provided limited information about behaviors that involved coordinated movements of body parts. Thus, although it was possible to measure whole-body movements of the animals, these approaches did not provide access to (for example) how an animal moved its legs or wings, or whether it touched another animal. Understanding these subtle movements is essential for uncovering the dynamics of these often highly choreographed interactions. The two studies highlighted here use recent advances in deep-learning-based computer vision to track the positions of multiple animals and estimate poses in different experimental paradigms (Fig. 1).

Multi-animal DeepLabCut (maDLC)[5] builds on earlier work, DeepLabCut[1], which is primarily used to track single animals. In maDLC, Lauer et al.[5] augment their approach