

**Figure 1 | Tibi and Wiens' observations<sup>1</sup> of the northern Tonga subduction zone.** Earthquakes in the subducted slab emit two types of seismic wave, P (solid black lines) and S (dashed lines), which reflect off the 660-km discontinuity below the slab and travel back up to the surface to seismic stations on Fiji and Tonga. Upgoing S waves also interact with the 410-km discontinuity and convert to P waves. The time lags between the direct P waves from the quake (not shown for clarity) allow the depth of the discontinuities to be estimated. The '660' behaves as expected. But the '410' seems to be depressed (purple; dashed where not observed, but inferred), not raised (white), near the slab. A conjectural flow of hot mantle material might explain the depression here, and a possible region of metastable olivine inside the slab (yellow) might bend the 410 into a downwards wedge-shaped form, making it more difficult to observe. (Modified from Fig. 2 in ref. 1.)

are therefore brittle. They are, in essence, cold fingers stuck into the warm mantle and so provide a deep-Earth laboratory to probe phenomena that occur where the mantle is cooler than normal. One such phenomenon is the behaviour of the worldwide velocity jumps in seismic-wave speed at depths of 410 km and 660 km. For 30 years these discontinuities have been attributed to pressure-induced changes in mantle minerals<sup>2</sup>, and, as such, have predictable responses to changes in temperature in subduction zones<sup>3–5</sup>. Tibi and Wiens, however, conclude that they don't behave entirely as expected.

The problem is sketched in Fig. 1. A cold subducted plate should cause a mineralogical change related to the 410-km discontinuity (that of olivine to wadsleyite) to occur at lower pressure, and so shallower in the mantle. Conversely, the change related to the 660-km discontinuity, that of the higher-pressure form of wadsleyite, ringwoodite, to perovskite and magnesiowüstite, should occur at higher pressure, and so deeper. Careful analysis of subduction-zone earthquakes yields the depths of the '410' and '660' discontinuities from the timing of secondary signals arising from the reflection and conversion of seismic waves at the discontinuity boundaries.

Tibi and Wiens<sup>1</sup> deployed portable seismographic stations on Fiji and Tonga, two islands that bracket the Tonga subduction zone in the

southwestern Pacific, and looked for the signals from the 410 and 660 discontinuities in their data. This was a challenging project — observing conditions on ocean islands are extremely tough because of ocean-wave-generated seismic noise and problematic logistics. What Tibi and Wiens found, however, was that the 660 went down as expected but the 410 didn't go up everywhere, in part contradicting the mineralogical-change explanation for the discontinuities.

What is going on? Possibly two things. One, discussed by the investigators, is that hot mantle affected by the warm, ascending material that causes 'hotspot' volcanism in Samoa, nearby to the north, might be drawn southwards by the subduction beneath Tonga, so heating the vicinity of the slab. If it does, in my view there must be terrific thermal gradients in the slab, because parts of it are still cold enough to cause earthquakes. But this hot mantle might narrow the uplifted part of the 410 discontinuity, making it difficult to observe, and depress the 410 outside the slab. The observed 25-km depression corresponds to about 280 °C hotter temperatures<sup>5</sup>, not impossibly high for hotspots. Yet somehow the 660 behaves appropriately, suggesting that the thermal perturbation does not extend that deep or arose only recently, after the deeper parts of the slab had slipped past the present warm flow.

Alternatively, although Tibi and Wiens don't favour this explanation, the transition from olivine to wadsleyite could be hindered by the low temperatures in the slab, and be pushed downwards in the form of a metastable olivine wedge in the slab<sup>6,7</sup>. If so, not all subduction zones behave in this way, because studies<sup>8</sup> of such a zone near Japan found the

410 raised to 350 km by cold slab temperatures.

Before giving up on the explanation that the 410-km discontinuity is caused by mineralogical change, it would be worth checking regional Tongan topography with underside reflections<sup>8,9</sup>. These are best investigated with observing stations that are at least 5,000 km distant, leading to reflection points that lie farther into the slab's interior because of the geometry. They are more sensitive to detecting topographic peaks than the methods used by Tibi and Wiens because reflection amplitudes remain strong for vertically travelling waves, whereas they decrease to zero for transmitted waves. This would settle the doubts about the dashed line in Fig. 1. On the other hand, the fading earthquake-limned grin of the hard-luck plate might be one of amusement at the head-scratching these observations are causing among Earth scientists. ■

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## CELL BIOLOGY

# Without a raft

Ben Nichols

**The spatial organization of signalling proteins in the cell membrane is often ascribed to lipid-based 'rafts'. But single-molecule tracking reveals that such organization probably arises by protein-protein interactions.**

Signal transduction — the relay of signals from outside a cell to inside — frequently involves bewildering patterns of interactions between several different types of protein at the cell surface. Work attempting to make sense of this complexity suggests that specific lateral organization of the interacting proteins in the membrane is key to their signalling functions. But how is such organization generated? Work by Douglass and Vale, published in *Cell*<sup>1</sup>, begins to provide some answers, and emphasizes the utility of recently developed single-molecule imaging techniques in addressing the dynamic properties

of signalling networks. Moreover, the authors' experiments directly address the controversy over the mechanisms that generate localized variations in the composition of the cell membrane.

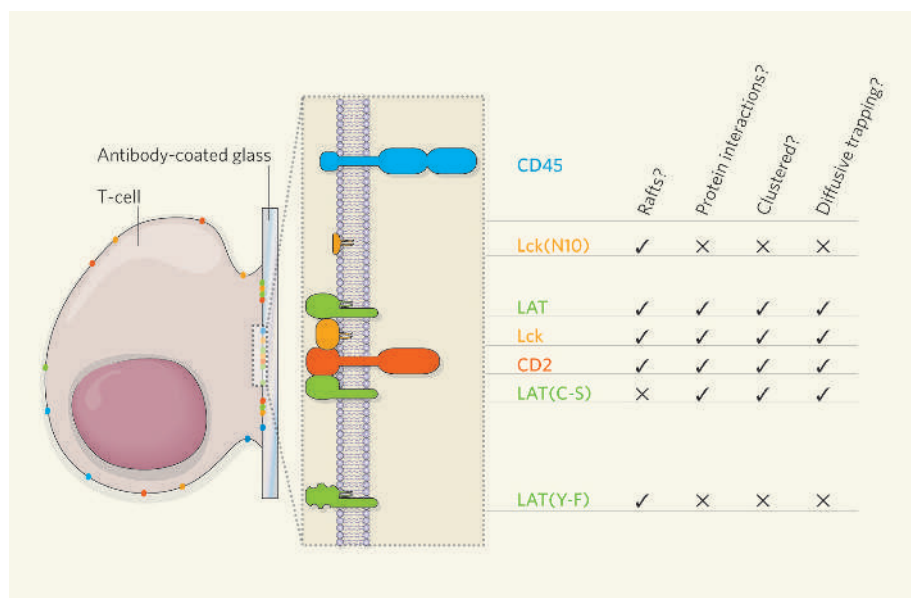
Simple mixtures of lipids in artificial membrane bilayers can segregate into regions that differ in the way the acyl chains of the lipids are packed together. This can spontaneously generate heterogeneity in the membrane, as lipids that prefer different local environments tend to separate out from one another. More-ordered acyl-chain packing is associated with the presence of increasing amounts of

cholesterol and sphingolipids — both found in natural membranes — and the resulting lipid domains tend not to be soluble in non-ionic detergents<sup>2</sup>. Detergent-insoluble fractions enriched for particular proteins and lipids can also be isolated from cells. Extrapolating from the artificial membrane data, it has been proposed that these detergent-resistant fractions might be derived from functional domains, or 'lipid rafts', in cell membranes, where self-organization of the membrane lipids leads to the recruitment of specific proteins<sup>3</sup>. This lipid raft hypothesis has received much attention and is certainly appealing, but the correlation between detergent resistance and domain formation *in vivo* is a topic of some debate<sup>4</sup>. Artificial membranes may not be good models for cell membranes that are rich in protein and have two asymmetric layers of hundreds of different types of lipid.

The case of signalling through T-cell receptors is particularly germane to this debate. At the start of an immune response, T cells are activated when antigen molecules bind to the receptors on their surface. Stimulation of T cells usually occurs when stable contacts — referred to as 'synapses' — form between the T cell and so-called antigen-presenting cells<sup>5</sup>. There is a striking degree of spatial organization within the synapse; for example, molecules involved in the adhesion of the interacting cells, and activators and inhibitors of the signalling cascade, segregate and take on highly specific patterns<sup>5,6</sup>. Moreover, because several of the proteins recruited to the T-cell synapse are found in detergent-resistant membrane fractions, various functions have been ascribed to lipid rafts in organizing these proteins during T-cell-receptor signalling<sup>7</sup>. This instance of a signalling machine in which the appropriate spatial distribution of its components is likely to be central to its function is a promising place to look for direct evidence of a physiological role for lipid rafts.

Douglass and Vale<sup>1</sup> adopted a widely used method for stimulating T cells — cross-linking the receptors using specific antibodies. But they modified it so as to be able to view the stimulated surface of the cell using either conventional confocal microscopy or total internal reflection imaging; the latter is a highly sensitive approach that can detect single fluorescent molecules in a narrow focal plane (Fig. 1). This remarkable technical achievement allowed individual fluorescent proteins involved in T-cell-receptor signalling to be tracked directly. Several of these proteins clustered together, for example the stimulatory co-receptor CD2, the adaptor protein LAT and the enzyme Lck; the negative regulator CD45 did not occur in the clusters.

LAT seems to play a prominent role in generating clustering, as the proteins do not group together in cell lines lacking LAT. By using a laser to photo-bleach a spot on the membrane, the researchers followed fluorescent molecules as they moved into the bleached area. This



**Figure 1 | Clustering to signal.** Incorporation of proteins into lipid rafts is not related to the dynamic clustering that occurs during activation of the T-cell receptor, according to work by Douglass and Vale<sup>1</sup>. The authors allowed T cells to settle on cover-slips coated with antibodies that cause localized activation of T-cell-receptor signalling. A combination of confocal microscopy and single-molecule imaging revealed that the proteins LAT, Lck and CD2 group together, and that these clusters exclude the negative regulator CD45. Freely diffusing LAT and Lck molecules can become transiently trapped in the cluster regions of the membrane. Clustering and this diffusive trapping require the protein-protein interaction domains of LAT and Lck (which are missing or defective in the LAT(Y-F) and Lck(N10) proteins). However, these actions are not affected by mutations that abrogate incorporation into lipid rafts (LAT(C-S)).

showed that there was a constant exchange of individual protein molecules in and out of clusters that were themselves stable over time. Finally, ingenious experiments tracking individual LAT, Lck and CD45 molecules relative to clusters containing CD2 revealed that these clusters can temporarily trap freely diffusing LAT and Lck, but not the negative regulator CD45.

Douglass and Vale's system provided an excellent opportunity to unravel the mechanisms that generate the spatial organization of signalling molecules (Fig. 1). A mutant of LAT, which lacks the acylated amino-acid residues required for incorporating the protein into lipid rafts<sup>8</sup>, showed identical clustering and diffusional trapping to normal LAT. Conversely, a LAT mutant lacking residues required for specific protein-protein interactions did not cluster or show diffusional trapping. Similar results were obtained with a truncated form of Lck that retains only the portions required for membrane association and raft incorporation. This mutant also did not cluster or show diffusional trapping.

These data do not support a link between raft incorporation (detergent resistance) and spatial organization during T-cell-receptor signalling. The authors propose instead that the clustering and diffusional trapping are best explained by a network of protein-protein interactions between the relevant signalling molecules. Although such a network remains to be characterized directly, previous studies corroborate the primary role of protein-

protein as opposed to protein-lipid interactions in T-cell-receptor signalling<sup>9</sup>. They also do not support the proposed link between raft incorporation and protein recruitment to membrane regions where the T-cell receptor has been activated<sup>10</sup>.

Now that our picture of protein dynamics during spatially organized signalling is beginning to reach single-molecule resolution, there seems no need to invoke the raft model to explain these dynamics. However, before summarily wielding Occam's razor, we should remember that model systems such as that used by Douglass and Vale do not fully replicate the properties of the T-cell synapse *in vivo*, and further subtleties doubtless remain to be investigated. ■

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