T-CELL SIGNALLING

A chicken or egg situation

Which comes first, the immunological synapse or T-cell receptor signalling? Current dogma dictates that signalling in T cells, and therefore T-cell activation, is a consequence of the formation of the immunological synapse — where a T cell contacts an antigen-presenting cell (APC) — which serves to cluster T-cell receptors (TCRs). But a report by Andrey Shaw's group challenges this model by presenting data indicating that TCR-mediated signalling occurs before mature synapses form.

Shaw and colleagues set out to determine exactly where the interaction between TCRs and the antigenic major histocompatibility complex (MHC)–peptide complexes of the APC occurs. They did this by spinning together antigenic-peptide-containing APCs and T cells, and analysing the localization of TCRs in the conjugates. TCRs of cells that were studied after a short spinning time (2–15 minutes) were detected at the edges of the synapse, but later (after 15 minutes) became concentrated in the centre. After 1 hour, though, no TCRs were found in the synapse. Changes in the staining pattern for the integrin LFA-1 confirmed that the synapse progressed from an immature (at 15 minutes) to a mature (30 minutes) state during this procedure.

So if the TCRs disappear from the mature synapse, what happens to TCR-mediated signalling? To address this, the authors studied the localization of active Lck and ZAP-70 (zeta-chain-associated protein kinase, 70 kDa) — two tyrosine kinases that are crucial in TCR signalling — in the conjugated cells. The results showed that phosphorylated Lck and ZAP-70 were only found at the edges of immature synapses and could not be detected in mature synapses, indicating that TCR-mediated signalling occurs well before the mature synapse has formed.

To see whether naive T cells could be efficiently activated by this seemingly short duration of TCR-mediated signalling, T cells that had been allowed to conjugate with APCs for defined lengths of time were analysed for cell-cycle progression. The authors found that T cells only divided after they had interacted with APCs for a minimum of 2 hours. These findings contrast with previous results, which propose that only long periods of TCR signalling (up to 20 hours) can activate T cells. What happens in the period after TCR signalling is attenuated, but before T cells commit to divide, is currently unclear, but might well involve other signalling pathways.

So what is the function of the immunological synapse if not to cluster TCRs? Although there are several hypotheses; for example, its involvement in polarized secretion, activation of other signalling pathways, or the endocytosis of TCRs, this is now the million-dollar question.

Katrin Bussell

References and links

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DNA METABOLISM

New link is old HAT

When DNA is under attack, the repair machinery moves in quickly. There's just one small problem — the damaged DNA is wrapped up as chromatin, which first needs to be unravelled (or 'remodelled'). A sensible solution would be to link the DNA repair machinery with chromatin-remodelling factors, but such a link has been elusive.

Reporting in *Molecular Cell*, Marc Tini and colleagues now describe the interaction of an enzyme involved in DNA repair — thymine DNA glycosylase (TDG) — with the transcriptional co-activators CBP or p300. Strikingly, the resulting complex is still active in DNA repair and transcription, suggesting that it functions in both processes.

Tini and co-workers detected the CBP–TDG complex by co-immunoprecipitation, GST (glutathione-*S*-transferase)-fusion-protein interaction assays, and gel filtration of fractionated HeLa cell extracts. They also showed colocalization of the proteins *in vivo*. CBP alone (tagged with a fluorescent protein) had a granular distribution in the nucleus and TDG alone showed a diffuse nuclear staining, whereas co-expression resulted in a distinctive macrogranular pattern for both proteins.

The authors next studied whether formation of the CBP–TDG complex affects the function of either protein. The complex showed high affinity for a G/T mispaired duplex oligonucleotide — a natural substrate for TDG — and was able to cleave it (an initial step in the repair process). Conversely, CBP retained its chromatin-remodelling (histone acetylase; HAT) activity in the CBP–TDG complex. Indeed, cotransfection with TDG led to a dose-dependent increase in the transcriptional activity of a GAL–CBP fusion protein, suggesting that TDG stimulates the transcriptional activity of CBP.

As CBP can acetylate both histones and non-histone proteins, Tini and colleagues wondered whether TDG might be acetylated by CBP. They found that it is, both *in vitro* and *in vivo*, and that acetylation leads to the release of CBP from the complex. Although acetylation did not affect the ability of TDG to bind or cleave mispaired DNA, it did prevent TDG from binding another component of the repair machinery, the apurinic/apyrimidinc endonuclease APE1. This suggests CBP-mediated acetylation could be involved in regulating DNA repair.

This is, say the authors, "the first example of a repair enzyme involved in detection of primary DNA lesions that interacts directly with… CBP/p300 and represents a new class of HAT substrate". So, by providing a chromatin-modifying activity at sites of G/T repair, the CBP–TDG complex could regulate the access of other components of the repair machinery to the DNA. Finally, these findings imply that mutations in *cbp* or p300 — which have been found in various tumours — could deregulate TDG-coupled DNA repair and so contribute to the genetic instability that is often associated with cancer.

Alison Mitchell

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WEB SITE

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