of CD8$^+$ cytotoxic T cells in mice to a peptide from an intracellular bacterial pathogen, *Listeria monocytogenes*, was similar whether or not the mice had been treated with an antibiotic 24 hours after infection. The antibiotic markedly reduced the number of bacteria, yet the response remained the same. Mercado *et al.* also transferred dye-labelled T cells, specific to *L. monocytogenes*, into mice; similar proportions of the T cells divided whether the infection was allowed to continue or was blocked by an antibiotic after 24 hours. Their results again indicate that once a T cell is stimulated it usually undergoes a complete programme of proliferation and specialization.

Kaech and Ahmed[2] add further insight into how the magnitude of a T-cell response relates to antigen load. They infected mice with different doses of *L. monocytogenes* that had been engineered to express a peptide from lymphocytic choriomeningitis virus (LCMV). They then injected the mice with dye-labelled T cells specific to the LCMV peptide. The T cells either followed the whole proliferative programme or did not divide at all; the higher the antigen dose, the more T cells responded. If the T cells were stimulated with antigen just briefly *in vitro*, they continued to divide and differentiate[2] (T cells may need to divide just once to be able to differentiate in this system[7]). All of these data[1,2,4], coupled with the observation that the peak in a T-cell response is often sharp rather than prolonged over several days[4–6], suggest that if a naive T cell is not stimulated during the first few days of infection, it may not respond at all.

Kaech and Ahmed also found that some of the stimulated T cells later became memory cells. The number of CD8$^+$ memory T cells has been shown to be proportional to the magnitude of the T-cell response before it is silenced[8]. These results are relevant to vaccination, as the development of a pronounced memory response could depend on the stimulation of a large proportion of naive, antigen-specific T cells early during infection.

T cells are clearly preprogrammed to some extent. It seems that, once stimulated briefly, T cells undergo at least 7 or 8 divisions, with generation times between 5 and 8 hours (refs 1, 2, 4), and that 12 or more divisions can take place before the immune response is silenced[5]. However, it is unlikely that the cells are programmed so precisely that they are oblivious to the effects of other external factors. Kaech and Ahmed[2] found that the division of CD8$^+$ T cells was blocked when interleukin-2, the receptor for the T-cell growth factor, was inhibited; it is noteworthy that CD4$^+$ T cells, the functions of which are compromised in AIDS, are rich sources of interleukin-2. Moreover, the persistence of antigen can continue to stimulate T-cell proliferation up to a point, but too much antigen can drive T cells into 'clonal exhaustion'[9].

Are any other functions of T cells programmed events? The mechanisms behind the death of T cells at the end of an immune response, and the slow division of memory T cells, have defied understanding. But these processes might similarly be explained by the existence of some autonomous, preprogrammed clock within the T cells themselves.

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Cell biology

Channels as enzymes

Michael D. Cahalan

The TRP family of ion channels is proving rather strange. The latest quirks of behaviour include a new enzymatic activity and second messenger, the ability to conduct magnesium ions, and an involvement in cell survival.

Simply put, ion channels are proteins that form pores in cellular membranes, conducting ions into or out of cells. Once thought to be involved merely in sending electrical signals from place to place in the body, ion channels are now known to have many functions in all types of cell. They allow a variety of positive or negative ions to enter or exit cells, and are regulated by many different molecules. The TRP channels, encoded by about 20 distinct genes, are possibly the strangest ion channels. For example, some are actually enzymes, catalysing not just the movement of ions, but also chemical reactions that couple to signalling and metabolic pathways within cells. On pages 590 and 595 of this issue, Scharenberg and colleagues[1,2] reveal yet more unusual features of TRP channels.

The TRP-channel family is undergoing a nomenclature upheaval at present, but all parties agree that three subfamilies can be distinguished on the basis of sequence gazing[3,4]. Each receptor folds up to crisscross the plasma membrane six times, with the amino and carboxy tails inside the cell. The transmembrane portions contribute to a cation channel, and the two ends have other functions. It is likely that four subunits assemble to form a functional channel, and that diversity is increased because different combinations of subunits can form the channels.

Members of the 'long TRP channel' (LTRPC) subfamily have unusually long amino and carboxy termini. The carboxy

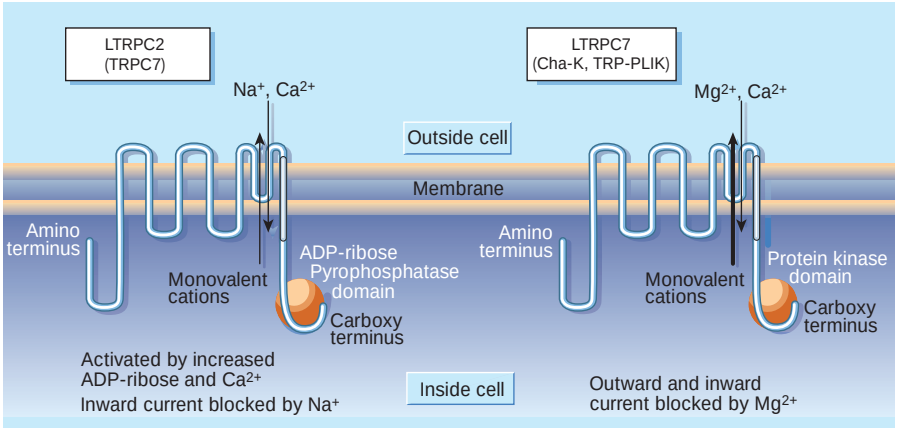

Figure 1 **The behaviour of two ion channels from the long-TRP-channel family. The alternative names of LTRPC7 and LTRPC2 (refs 1, 2) are shown, along with their enzymatic activities and ion-permeation properties. The inward current through LTRPC7 is carried by calcium or magnesium ions, and is small compared with the outward current carried by monovalent cations — in other words, LTRPC7 is a strong outward rectifier[1]. LTRPC2 allows inward current (carried by the combination of sodium and calcium ions) or outward current (carried by monovalent cations) to flow equally[2].**

termini contain domains that have enzymatic activity (Fig. 1). LTRPC7, for example, has an unusual protein kinase domain, which can catalyse the transfer of phosphate from adenosine triphosphate (ATP) to several substrates[5], including LTRPC7 itself.

Although the natural activators of LTRPC7 remain unknown, Runnels *et al.*[5] conclude that its kinase activity is strictly required for its channel function. They found that the addition of ATP to the inside of cells increased the flow of ions through the channel, suggesting that ATP (and, by inference, the kinase domain) is needed to open the channel.

This conclusion is challenged in one of the new papers[1]. The discrepancy centres on the relationship between Mg^{2+} ions and ATP. Nadler *et al.*[1] find that the channel's activity is suppressed by Mg^{2+} ions, whether free or bound to ATP. They suggest that the addition of ATP by itself would lower the concentration of free Mg^{2+} ions. So, the channel activity observed by Runnels *et al.* in the presence of ATP might be explained by a fall in the concentration of free Mg^{2+}, without needing to invoke a role for LTRPC7's kinase activity. Other results, obtained with a hydrolysis-resistant analogue of ATP[2], provide further evidence against phosphorylation-dependent gating of LTRPC7. But this is a debate that is likely to rumble on.

As the authors show in the second paper[2], the long TRP channel LTRPC2 also has an enzymatic domain at its carboxy tail. This domain enables the protein to remove the terminal ribose-5-phosphate moiety of ADP–ribose. ADP-ribose also activates the channel, suggesting that, in this case, the enzymatic activity might actually be involved in opening or closing the channel. Once activated, the channel can remain open for a prolonged period even after ADP-ribose has been removed — a molecular memory worth pursuing further.

The LTRPC7 channel needs to be regulated properly for cells to survive: when Nadler *et al.*[1] either overexpressed or knocked out LTRPC7 in different cell types, the cells died. Under normal conditions, LTRPC7 is a strong outward rectifier[1], meaning that the inward current (carried by Ca^{2+} or Mg^{2+} ions) is smaller than the outward current (carried by monovalent cations, such as K^+). It seems plausible that, when LTRPC7 is overexpressed or knocked out, the levels of Ca^{2+}, Mg^{2+} or possibly other polyvalent cations might become unbalanced. Moreover, both LTRPC2 and LTRPC7 might be affected by ischaemic conditions, which result from oxygen depletion. Intracellular ATP-bound Mg^{2+} blocks both inward and outward currents through LTRPC7 (ref. 1), and Na^+ selectively blocks inward currents through LTRPC2 (ref. 2). In response to ischaemia, levels of Na^+ inside cells would rise and levels of Mg^{2+}–ATP would fall. So ischaemia might block inward currents through LTRPC2, and activate inward and outward currents through LTRPC7.

These papers[1,2] might also have a bearing on one of the major puzzles in ion-channel biology: the identity of 'store-operated' Ca^{2+} channels. When a cell's Ca^{2+} stores are emptied in response to some signal, Ca^{2+} enters the cell, because Ca^{2+} channels in the plasma membrane open. The Ca^{2+}-release-activated Ca^{2+} (CRAC) current found in the immune system is one of the best-characterized store-operated currents, yet the molecular identity and mechanism of activation of the CRAC channel have been elusive. However, TRP4 and CaT1, which are both members of the TRP family, are leading contenders[6–8].

LTRPC2 and LTRPC7 are also expressed in immune cells[1,2], and Nadler *et al.* and Perraud *et al.* describe currents that resemble those through these channels in some types of immune cell. These results imply that the conditions used to investigate CRAC and other store-operated channels in immune cells might have unintended effects on cellular physiology, by perturbing cellular ions and metabolites during whole-cell recording. My group recently characterized single channels in human T cells activated under conditions that deplete Ca^{2+} stores passively (using strong Ca^{2+} buffers). These channels resemble CaT1 channels in some aspects and LTRPC7 channels in others[9]. The molecular identity of CRAC channels is yet to be pinned down, but further characterization of single channels and the development of molecules that block specific channels would help in the investigation.

As a final note, TRP channels are important in disease as well as health. For example, the levels of specific LTRPC-family members change during the progression of tumours such as melanoma and prostate cancer[10,11]. Before we can understand the role of these channels in disease, we will need to have a clearer understanding of how they function as molecular devices. Here, too, the development of selective channel blockers — both as experimental tools and as potential treatments — should be a high priority. ■

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Daedalus

Four feet, one mouth

In days gone by, by-products were a valuable asset. The LeBlanc process for manufacturing sodium carbonate survived for decades on its by-products. These days they merely complicate marketing; demand for main products and by-products can fluctuate unpredictably. But one industry, farming, is stuck with by-products. Every liver brings one heart, unavoidably. And now that foot-and-mouth disease has made even farmers question their livelihood, Daedalus hopes to exploit modern biochemistry.

He points out that stem-cell research allows any number of cells of any sort to be made. Cells very close to their origin can develop into anything — new brain cells, for example. And by sticking with non-human tissue, all sorts of ethical dilemmas can be avoided. Daedalus recalls how rapidly a damaged liver can regenerate. Suppose, he says, a liver culture were fed with stem cells influenced by signalling chemicals and environment to develop as liver cells. You could haul an endless liver slowly from the dish, overcoming the problem of having a by-product.

So DREADCO biochemists are exploring Daedalus' theory that stem cells can be made in bulk by developing new cells in a very 'young' medium. In due course it should be possible to haul a cow leg, say, or a multi-jointed leg, from a dish whose cells are primed to develop in a leggy manner. Much of the by-product problem would be overcome, although there would still be a fixed amount of skin (for shoes) and bone (for soup and gelatine film). Ultimately all animal husbandry, a cruel and inefficient process, will be replaced by painless tissue culture.

Plants will be exploited too. Daedalus reckons that rhubarb grows because its stalk gains glucose diffusing down from its leaf. If so, an endless rhubarb stalk could be made by pulling a natural one while feeding it glucose. Again, the by-product problem (poisonous leaves) would be neatly overcome. The DREADCO team is now exploring the animal and vegetable kingdoms in search of suitable species. Rhubarb is possible; so is potato (a large tubular tuber could be pulled from the photosynthesizing fronds), and so are snake and eel, although most animals are badly designed for the job. Ultimately biomass, now seen as an answer to our energy needs, could take its place at the dinner-table as well. But probably synthesized liver, leg joints and rhubarb pie would be more welcome than pure masses of animal or vegetable cells. David Jones