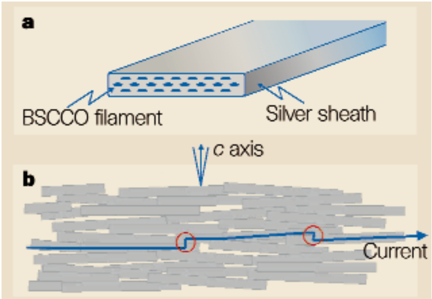

Figure 1 **Sketches of** (a) **a standard industrial superconducting tape, with 'BSCCO' filaments embedded in a matrix of silver, and** (b) **the cross-section of a single BSCCO filament, tracing a flow of current through the assembly of crystalline grains.**

wire. These wires are cut and re-stacked for a series of additional drawing, rolling and heat-treatment steps, leading to the final multi-filament oxide-powder-in-tube (OPIT) tapes (Fig. 1a). The silver matrix is chemically compatible with BSCCO, and endures the necessary high-temperature processing in oxygen. The fine BSCCO filaments, ~100 μm wide by only a few μm high, tolerate an acceptable amount of bending, like the fine glass filaments in flexible fibre-optic cables. Most importantly, the processing aligns the crystalline BSCCO grains within a filament, so that the *c* axes are perpendicular to the tape plane (to within 10–15°). Within a filament, electric currents can thus flow from grain to grain predominantly within the CuO_2 sheets (Fig. 1b).

Why is the limiting current density still lower than observed in single crystals and epitaxial films? Cai *et al.*[1] have used magneto-optical imaging of magnetic flux penetration to show that many filaments are not active at all, owing to the intrusion of cracks from the tape edges. These gross defects are probably remnants of deformation and heat treatment, and might be cured by further refinements in processing. But the authors' measurements also revealed signs of some *c*-axis conduction, implying the existence of local barriers that block basal-plane conduction, forcing the weak, *c*-axis tunnel-currents to take over (Fig. 1b).

These barriers may be microstructural flaws, such as voids, cracks or second-phase defects. Or, more ominously, they may be intrinsic weak links, where the misalignment between adjacent grains is too large to accommodate basal-plane current transfer. Indeed, supercurrent transport across a grain boundary can be greatly suppressed[2] for misalignments greater than ~10°. This is true even for the case where the *c* axes are perfectly parallel, and the adjacent crystals are merely misaligned in-plane (so-called [001] tilt-grain boundaries). This handicap may be intrinsic to HTS because of their low carrier density and strong sensitivity to structural disorder[3–5].

So, what are the prospects for improving BSCCO OPIT tapes? Refinements in thermo-mechanical processing — such as additional mechanical constraints, or higher-temperature rolling — should prevent filament cracks, and could also improve *c*-axis texture and filament microstructure. But processing trade-offs might persist, with competition between good interior filament properties and the elimination of gross defects near the tape edge. More seriously, at the moment there is no feature of OPIT tape processing that can lead both to *c*-axis and to in-plane grain alignment. Such biaxial alignment would give long conductors single-crystal-like properties, and work is under way to develop second-generation HTS wires by growing thin unbroken coatings of $YBa_2Cu_3O_7$ on flexible metal tapes[6].

The obstacles may appear daunting, but the specifications of OPIT tapes have continued to improve steadily with time[7]. Although a ceiling awaits, elimination of the cracks, and improvements of a factor of two in the filaments would yield overall current densities of ~50 kA cm^{-2} at liquid-nitrogen temperatures. That is good enough for economically and environmentally appealing low-magnetic-field applications, such as underground transmission lines and power transformers. For this, the development would take about six years, but coated conductors may provide a leap in capability well before then. Indeed, commercial development of HTS is on schedule when compared with the histories of other high-technology products, such as integrated circuits and optical-fibre communications[7]. There is every reason to be optimistic that the current problems will be solved. □

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Calcium signalling

Oscillation, activation, expression

Jacopo Meldolesi

Increased concentrations of free Ca^{2+} within the cytosol of living cells lead to the activation of arrays of both rapid and sustained events. These, and the events that cause them, can vary considerably under different conditions. As well as stable increases, most cells — both excitable and non-excitable — can develop oscillations in the concentration of cytosolic Ca^{2+}. These trains of Ca^{2+} spikes occur at much lower frequencies than those of membrane action potentials. But how do cells interpret these rises in the concentration of Ca^{2+}?

Oscillations in the concentration of cytosolic Ca^{2+} have attracted considerable attention, and hypotheses of frequency coding were proposed a decade ago[1]. But the experimental evidence has remained weak[2], and the physiological significance of the phenomenon is still questioned. On pages 933 and 936 of this issue, however, Dolmetsch *et al.*[3] and Li *et al.*[4] report highly original experiments in which oscillations were generated by new procedures, bypassing the complexities of cell heterogeneity and activation of surface receptors. The results clearly show that oscillations and their frequencies can be specific for gene activation, not only in terms of efficiency, but also of selectivity. Nothing of this kind has ever been demonstrated before, and these reports open a new field in Ca^{2+} signalling.

Li *et al.*[4] report a great technological success — the development (by a 14-step synthesis) of a modified version of inositol-1,4,5-trisphosphate, Ins(1,4,5)P_3, the second messenger that normally causes the release of intracellular Ca^{2+} stores. The newly developed molecule is not only membrane permeant but also caged, requiring photolysis (by ultraviolet light) for activation. It can therefore be loaded into intact, healthy cells, without inducing any effects until the Ins(1,4,5)P_3 is released. The unique advantage of this procedure is that the release of Ca^{2+} from intracellular stores (Fig. 1a, overleaf) can be modulated at will, in amplitude and in frequency, by varying the duration and frequency of the ultraviolet flashes.

The authors found that in RBL-2H3 basophilic leukaemia cells, trains of short (0.3–1.5-second) ultraviolet pulses, spaced 0.5–8 minutes apart, induced oscillations in the concentration of cytosolic Ca^{2+}. They then used this system to study, at the single-cell level, the activity of the Ca^{2+}-dependent transcription factor, nuclear factor of activated T cells (NF-AT). Activity of the gene was monitored using a procedure that the same group had developed[5], based on transfecting RBL-2H3 cells with a reporter construct that drives the expression of β-lactamase. Li *et al.* found that oscillations in the concentration of intracellular Ca^{2+} were more effective than a single, prolonged increase, provided the period of each oscillation was roughly one minute — slower and faster frequencies were less efficient. In

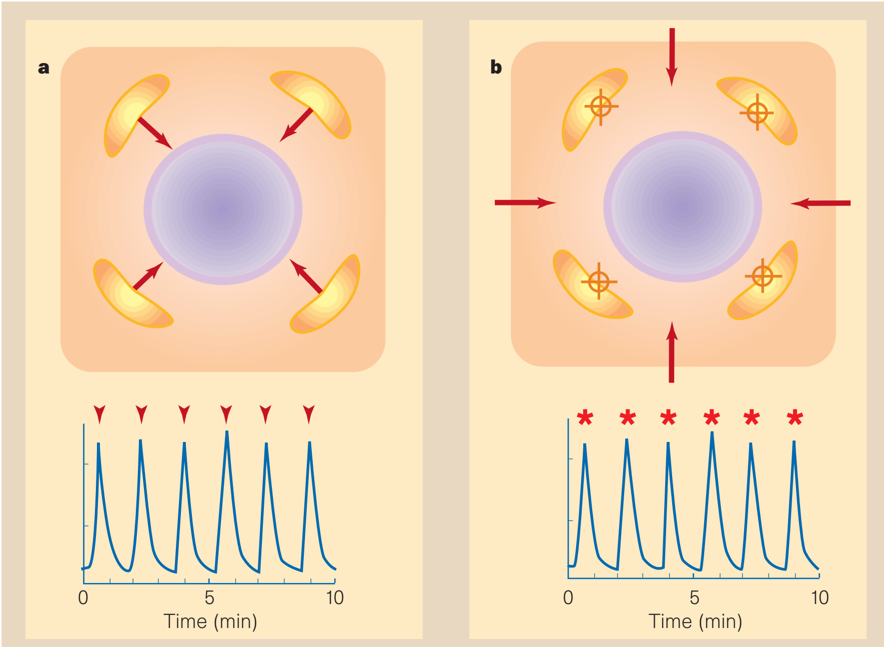

Figure 1 **New procedures for generating homogeneous oscillations in the concentration of cytosolic Ca^{2+}.** a, **The method developed by Li *et al.*[4]. Cells are loaded with a caged version of inositol-1,4,5-trisphosphate, which is released by rhythmic flashes of ultraviolet light (arrowheads). Activation of the corresponding receptor in the endoplasmic reticulum leads to the release of Ca^{2+} to the cytosol (arrows) in an oscillating manner.** b, **The method developed by Dolmetsch *et al.*[3]. Irreversible blockade of SERCA, a Ca^{2+} pump in the endoplasmic reticulum, by thapsigargin (⌖) prevents accumulation of Ca^{2+} within intracellular stores, so these become depleted. The resulting increase in the permeability of the plasmalemma to Ca^{2+} (due to activation of the store-dependent CRAC channels; arrows) is used to generate oscillations in the concentration of cytosolic Ca^{2+}. These oscillations are driven by rises in the concentration of Ca^{2+} in the incubation medium (asterisks).**

the case of the faster frequencies, this may have been due to desensitization of the $Ins(1,4,5)P_3$ receptor[4].

Dolmetsch *et al.*[3] took a completely different approach — rather than stimulating $Ins(1,4,5)P_3$-sensitive Ca^{2+} stores, they depleted them. Jurkat T cells were exposed to thapsigargin, which irreversibly blocks the specific Ca^{2+} pumps known as sarcoplasmic–endoplasmic reticulum Ca^{2+}-ATPases (SERCAs). This resulted in activation of the store-operated Ca^{2+} (CRAC) channels in the plasmalemma, leading to a stable increase in permeability of the cells to Ca^{2+}. Using this procedure, oscillations of controlled amplitude (generated by rapidly changing the concentration of Ca^{2+} in the incubation medium) were dynamically clamped. So, these oscillations were generated at the cell surface, without any involvement of intracellular receptors (Fig. 1b).

Dolmetsch *et al.* used this system to compare the effects, on NF-AT-induced gene expression, of oscillations and stable responses of similar average amplitude. They then looked, in parallel, at the activation of three Ca^{2+}-activated transcription factors — NF-AT, Oct/OAP and NF-κB. In each case, activation was monitored by the expression of reporter genes driven either directly or through the promoters of specific cytokines. Again, oscillations proved to be more efficient than stable increases — but only when the average concentration of cytosolic Ca^{2+} remained below ~300 nM. Moreover (and even more excitingly), whereas the three transcription factors were activated in parallel with stable increases in the concentration of Ca^{2+}, such parallel activation was observed for the oscillations only if they occurred with a period of 400 seconds or less. With oscillations of a lower frequency, only NF-κB seemed to be stimulated. These low-frequency oscillations are thought to be triggered by intercellular signalling events, so they are important in cell physiology.

How can these results be explained? Activation of the three transcription factors studied by Dolmetsch *et al.* is mediated by a Ca^{2+}-dependent phosphatase called calcineurin. The transcription factors exist as complexes in the cytoplasm, and they are dephosphorylated by calcineurin. This induces dissociation of the complexes, followed by migration of the active subunits to the nucleus[6,7]. The ensuing expression of specific genes is not controlled directly by Ca^{2+}, but it keeps going as long as the transcription factor remains in the nucleus. During oscillations, therefore, the work of transcription factors does not decline with the decreasing concentration of cytosolic Ca^{2+} — it can persist for longer. Moreover, because NF-AT returns to the cytoplasm

100 YEARS AGO

Striking is the difference in appearance between a Solpuga fasting and a Solpuga full fed. In the former the abdomen shrivels up, the segments shrinking one within another like the several pieces of a half-closed telescope; in the latter the expansion is carried to such an extent that the distended abdomen much resembles a short thick sausage, far surpassing in size and weight the rest of the body and limbs. This is brought about by the imbibition of water and of the fluid and semi-fluid tissues of their prey. In support of their water-drinking propensities, the following passage, written by the Soudan war correspondent to the *Standard* (October 19, 1897), may be cited: "One day in my tent [at Kerma] I heard a rustle like that of a silk dress. A big, ugly, yellow hairy beast, with nippers like a crab, was moving fast as a mouse over the moist ground near the zeer [porous water jar] in the corner of my tent. At last he settled down to suck the water from the sides of the jar." The writer of the passage just quoted had previously spoken of this animal as the "famous abu-shabat, the terror of the Soudan in the way of spiders, as large as your hand and ten times more venomous than a scorpion."

From *Nature* 28 April 1898.

50 YEARS AGO

Mainini has described (*Semana Medica*, 64, 337, March 1947) a pregnancy test in which, following injection of pregnancy urine into the male toad (*Bufo arenarum* Hensel), liberation of spermatozoa into the urinary bladder occurs within three hours; the same animal can be used again after five days. Drs. Octavio Rodrigues Lima and Oswaldo Gelli Pereira, of the Obstetrical Clinic, Medical School of Rio de Janeiro, University of Brazil, state in a communication submitted to the Editors that they have confirmed this work, using as test animal the male toad (*Bufo marinus*), as the species used by Mainini was not available to them. ... Sixty tests were carried out with the urine of amenorrhœic women. Positive results were found between half an hour and two hours, and were confirmed clinically in all cases. The test appears to be simple, economical and reliable.

From *Nature* 1 May 1948.

rapidly (~ one minute) after rephosphorylation by a specific kinase, whereas NF-κB returns much more slowly (> 16 minutes after a single Ca^{2+} spike)[4,8], the persistent activity of NF-κB during low-frequency oscillations can be explained.

The work of Dolmetsch *et al.*[3] and Li *et al.*[4] will make available to all Ca^{2+} laboratories direct procedures for generating oscillations in the cytosolic concentration of Ca^{2+} (Fig. 1), bypassing the drawbacks of heterogeneity due to cell and receptor variability. These studies will also enable us to begin to answer questions related to selectivity of Ca^{2+} signalling, and to look for subtle mechanisms of specificity in other Ca^{2+} responses. The general question is whether other signalling pathways are interpreted by cells — in terms of both the intensity and frequency of the message — as shown here for calcineurin-dependent gene activation. □

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Environmental science

Nitrogen oxides and tropical agriculture

A. F. Bouwman

Since 1992, and the appearance of the first scientific assessment of the Intergovernmental Panel on Climate Change[1], a great deal of evidence has accumulated showing that agricultural activities, especially application of fertilizers and animal manure, have resulted in increased emissions of nitrous oxide (N_2O) and nitric oxide (NO) to the atmosphere[2]. The reasons why we should care about this are that N_2O is one of the so-called greenhouse gases, constituting 6% of the anthropogenic greenhouse effect[2], and also contributing to the depletion of stratospheric ozone; NO, too, is an important player in atmospheric chemistry, for it participates in regulating the oxidant balance in the troposphere.

These assessments are based on evidence from temperate areas. But what of the tropics? Writing in *Global Biogeochemical Cycles*[3], Veldkamp, Keller and Nuñez discuss measurements[3–6] of nitrogen oxide fluxes from soils in tropical agricultural systems and argue that fluxes in such systems may be much higher than those generally observed in temperate regions. They also point out that estimates of these fluxes depend less on fertilizer composition, as has sometimes been assumed, and more on the conditions in the soil concerned and the timing of treatment.

Release of nitrogen oxides occurs in soils during both biological nitrification and denitrification, and during chemical denitrification. Biological denitrification is the reduction of nitrate (NO_3^-) or nitrite (NO_2^-) to gaseous nitrogen oxides and molecular N_2 by essentially anaerobic bacteria. Both N_2O and NO can be produced and consumed by denitrification. Chemical denitrification is the reduction of NO_2^- or NO_3^- by chemical reductants, while nitrification is the biological oxidation of ammonium (NH_4^+) to NO_2^- or NO_3^- under aerobic conditions.

After NO is emitted from the soil, it is often rapidly oxidized to nitrogen dioxide, NO_2, which is then readily absorbed onto leaf surfaces if a tree or shrub canopy is present, reducing the amount of NO and NO_2 escaping from the soil–plant system into the atmosphere. This reabsorption process is, however, less important in agricultural crops and grasslands than in dense forest canopies.

The regulation of the production and consumption of N_2O and NO by nitrification and denitrification in soil has been described by a 'hole-in-the-pipe' model (Fig. 1). A second model describes the effect of the soil-water status (Fig. 2). Veldkamp *et al.*[3] found that the relationship between the water-filled pore space (WFPS) and N_2O fluxes observed in three studies[3,4,6] in tropical systems was in close agreement with the theoretical model (Fig. 2), supporting the theory that in these systems NO stems primarily from nitrification and N_2O from denitrification.

Turning to look at differences in the crop systems, they found that, in tropical pastures with a fertile loamy soil developed from volcanic ash, the fraction of fertilizer nitrogen lost as N_2O (6.8%) and NO (1.3%) was much higher than the loss rates generally observed in temperate agricultural lands (about 1% and 0.5% for N_2O and NO, respectively[7,8]). On a banana plantation with similar soil, the loss rates for N_2O and NO were 1.3–2.9% and 5.1–5.7%, respectively[6]. In a fertilized sugar-cane field in Hawaii[4] and fertilized pastures with well-aerated soils in

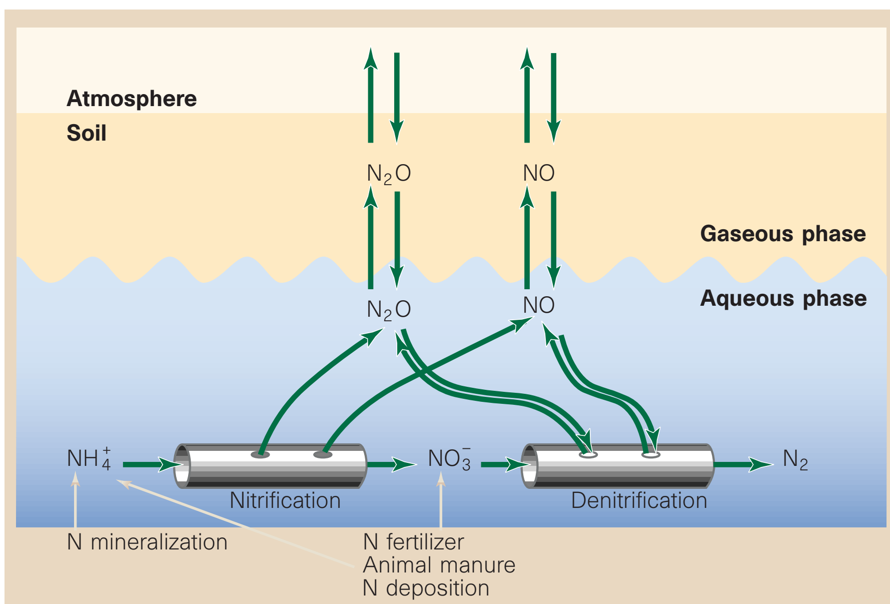

Figure 1 **'Hole-in-the-pipe' model of the regulation of trace-gas production and consumption by nitrification and denitrification[12]. In this model, gas production and exchange with the atmosphere depend on: ● Factors controlling the amount of nitrogen flowing through the pipe (that is, those affecting denitrification and nitrification rates, mainly nitrogen availability and temperature). ● The size of the holes in the pipe through which nitrogen gases leak. Size is regulated by factors controlling the partitioning of the reacting nitrogen species to NO, N_2O or more reduced or oxidized forms, while the rate at which nitrogen moves through the pipes determines the importance of the leaks. ● The properties of the soil and length of the path between the production site and the open air. Before escaping from the soil to the atmosphere, the nitrogen gases diffuse through the soil pore system, where NO in particular may be taken up by plants or microorganisms.**