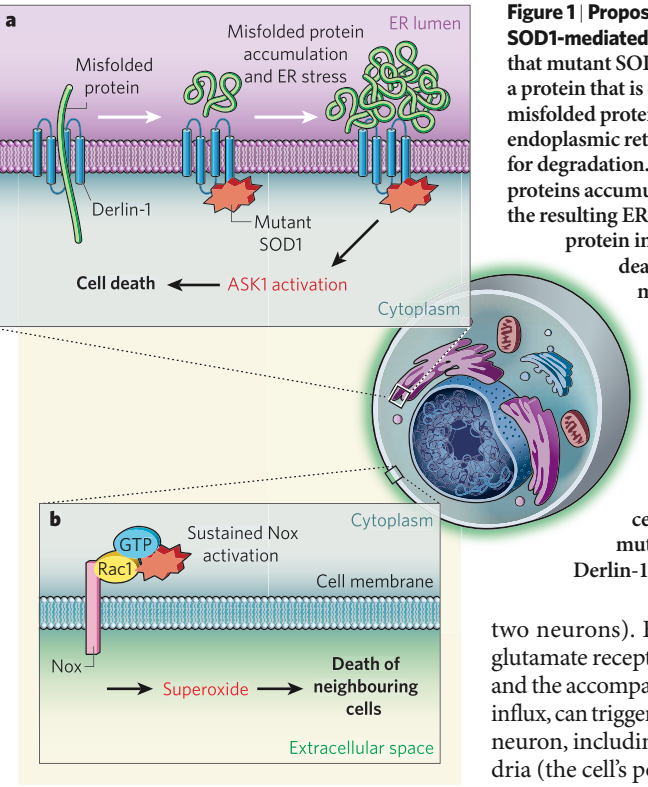

Figure 1 | Proposed mechanisms of toxicity in SOD1-mediated ALS. **a**, Nishitoh *et al.*[1] show that mutant SOD1 interacts with Derlin-1, a protein that is essential for transporting misfolded proteins from the lumen of the endoplasmic reticulum (ER) to the cytoplasm for degradation. Consequently, misfolded proteins accumulate in the ER lumen, and the resulting ER stress activates ASK1 — a protein involved in programmed cell death — by an unidentified mechanism. **b**, Near the cell membrane, mutant SOD1 also interacts strongly with Rac1 (ref. 6), keeping the Nox enzyme active. The resulting increased production of the superoxide radical, and its release into the extracellular space, is toxic to neighbouring cells. The precise cells in which mutant SOD1 interacts with Derlin-1 or Rac1 are unknown.

such as microglia — to kill bacteria and other pathogens. It emerges that the association of normal SOD1 with Rac1 is part of a tightly regulated mechanism that, under chemically reducing conditions, activates Nox. But mutant SOD1 interacts with Rac1 with a higher affinity than normal, 'locking' Nox in its active, superoxide-producing form, even under oxidizing conditions. This results in a tenfold increase in superoxide production and its release into the extracellular space (Fig. 1b).

Paradoxically, therefore, instead of carrying out its normal job of removing intracellular superoxide, mutant SOD1 might increase extracellular levels of this radical and so damage motor neurons and other cells. A Nox inhibitor improved survival in a SOD1 mutant mouse almost exclusively by delaying the onset of ALS[7]. As the presence of mutant SOD1 in motor neurons, but not microglia, is one of the factors that determine the timing of disease onset, this finding suggests that the interaction between mutant SOD1 and Rac1 triggers disease by activating a Nox variant (Nox1) found in motor neurons, rather than another variant of this enzyme (Nox2) that occurs in microglia.

So, are ER stress[1,6] and the increased production of superoxide[7] — both potential triggers of death for motor neurons and neighbouring cells — the whole story of the molecular events underlying ALS? Almost certainly not. Observations in SOD1 mutant mice and in tissue samples from patients with sporadic ALS suggest that one contributory factor to the damage is excitotoxicity (excessive firing of motor neurons that occurs when the stimulatory neurotransmitter glutamate is not rapidly

two neurons). Excessive stimulation of the glutamate receptors on neuronal membranes, and the accompanying increase in calcium-ion influx, can trigger a cascade of toxic events in the neuron, including damage to both mitochondria (the cell's powerhouses) and the ER (the main reservoir of intracellular calcium). Loss of the glutamate transporter EAAT2, a synaptic 'vacuum cleaner' for glutamate, from neighbouring astrocytes contributes to this phenomenon[8], but a direct role — if any — for mutant SOD1 in this mechanism remains unclear.

Mutant SOD1 has also been proposed[9] to interact directly with chromogranins (components of vesicles secreted by neurons) and to co-secrete with them. This in turn may trigger damage to motor neurons and neighbouring cells.

And there is more. Mitochondrial dysfunction has long been implicated in the damage to motor neurons seen in ALS. Misfolded mutant SOD1 is deposited on the cytoplasmic face of the outer mitochondrial membrane in cells of the spinal cord[10]. Moreover, mitochondrial dysfunction in motor neurons[11] carrying SOD1 mutations is associated with the release of cytochrome *c*, a potent trigger of cell death. Finally, mutant SOD1 provokes oxidative stress in astrocyte mitochondria[12], which could accelerate death in neighbouring neurons.

With all these potential contributors, the proposed pathways of pathogenesis in inherited ALS might strike some as "curiouser and curiouser", much as Alice proclaimed of the strange happenings in Lewis Carroll's *Alice's Adventures in Wonderland*. Among the biggest challenges will be to distinguish mechanisms that cause the disease from those that are only epiphenomena. In our view, the most likely possibility is that every set of observations discussed here is partly right, with age-dependent, selective toxicity requiring a convergence of damage to motor neurons and non-neurons alike. So stay tuned: the main pieces of this puzzle are yet to be revealed. ■

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EARTH SCIENCE

Volcanic cause of catastrophe

Timothy J. Bralower

From the timing, it looks as if an episode of marked oceanic oxygen deficiency during the Cretaceous was the result of undersea volcanism. Studies of such events are relevant to the warming world of today.

About 93 million years ago, Earth was shaken by an immense episode of volcanism. Massive piles of highly fluid lava accumulated under the seabed, forming much of the present-day Caribbean region in a geological heartbeat. On page 323 of this issue[1], Turgeon and Creaser argue persuasively that these eruptions triggered an episode of oceanwide anoxia that led to mass extinctions. Moreover, at this time and large reptiles roamed northern Canada. So this new work can provide valuable lessons about the way Earth responds to perturbations akin to those it is experiencing now.

The stack of lava flows that formed the Caribbean tectonic plate is known as a large igneous province (LIP)[2,3]. Other LIPs altered Earth's history by causing profound changes in the composition of the atmosphere: by

greenhouse warming; by altering the chemistry of sea water and ocean circulation; and by disrupting the global carbon cycle[4]. A LIP in Siberia that erupted 250 million years (Myr) ago led to the largest mass extinction ever, at the end of the Permian period[5]. Another event 120 Myr ago, the formation of the massive Ontong Java Plateau in the western Pacific Ocean[6], led to extinctions of ocean plankton. Both of these LIPs caused the ocean circulation to slow, decelerating the cycling of carbon and oxygen, and ultimately leading to highly toxic, anoxic conditions on the sea floor.

Episodes of anoxia, known as oceanic anoxic events (OAEs), have occurred periodically during Earth's history, but none was more severe than that which occurred 93 Myr ago, during the Cretaceous period[7,8]. This OAE caused the extinction of large clams known as inoceramids and tiny protists called foraminifera that lived on the sea floor. Profound changes in ocean circulation also led to the production and preservation of enormous quantities of marine organic material that was subsequently transformed into oil during its burial. But the ultimate cause of the OAE has proved elusive. There is strong evidence that warming was involved, implicating greenhouse conditions[9]. Unusual metal enrichments in rocks deposited during the OAE suggest a link to volcanism[10,11], but rocks that are organic-rich are often metalliferous as well, so this link remains unconfirmed.

The Caribbean lavas are now deeply buried in the ocean or found in mountain belts in places such as Haiti, where they have been exhumed during tectonic activity. Their eruption history is not as well known as that of the Siberian and Ontong Java LIPs, which are more widely sampled. The lavas' ages, measured using radioactive isotopes, span the interval 87–95 Myr ago, with suggestions that a large pulse occurred 93–94 Myr ago[12]. But given that the Caribbean is hundreds of thousands of cubic kilometres in extent, it is impossible to determine its eruption history with only a few age estimates. Instead, geologists must rely on other chemical signals in sedimentary rocks that provide continuous proxies for volcanic intensity through time.

Turgeon and Creaser[1] used a sensitive proxy for volcanism based on two isotopes of osmium, ^{187}Os and ^{188}Os. The main sources of osmium to the ocean include detritus from rivers, volcanism and material from outer space such as meteorites. Thus, the osmium isotope composition of sea water is a measure of the weathering of the continents, volcanic activity and extraterrestrial input[13]. The abundance of both osmium isotopes in sea water is low, so their ratios adjust rapidly to changes in input.

Turgeon and Creaser's careful analysis of these isotopes in sedimentary rocks from drill cores off the coast of South America, and from mountains in Italy, provides clear evidence of a perturbation that immediately preceded the

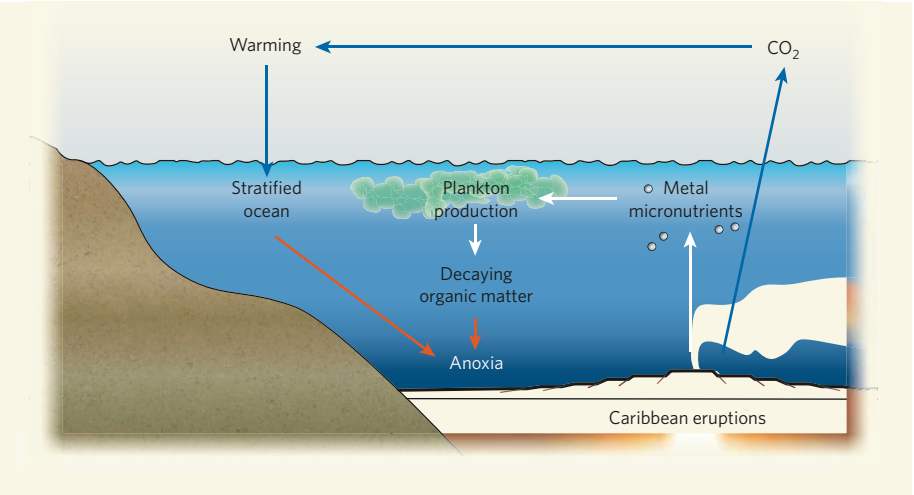

Figure 1 | Volcanism, oceanic anoxia and global warming. Turgeon and Creaser[1] provide good evidence for a causal connection between the extensive eruptions in the Caribbean region 93 million years ago and the oceanic anoxic event of that time. But how might they have been connected? One possibility is that the volcanism seeded the upper ocean with metal micronutrients, increasing phytoplankton production, which in turn led to increased oxygen use during the decay of organic matter. Another, not mutually exclusive, possibility is that a consequence of the global warming stemming from volcanically produced CO_2 was a more stratified ocean, in which oxygen delivery to deep waters became restricted.

unmistakable increase in the osmium contribution from a meteoritic or volcanic source. In the absence of other evidence for an extraterrestrial impact at this time, the data clearly point to a volcanic episode. And given that the shift in osmium isotopes suggests a factor of 30–50 increase in the osmium flux to the oceans, that episode was evidently on a huge scale. Although there are several candidates, the only LIP close to this age that is large enough to have caused this type of perturbation is in the Caribbean.

The formation of the Caribbean is of great interest to geologists, but it also has much broader implications. Abrupt warming events in the geological record are of great significance for scientists working on modern global warming. The ancient episodes provide a complete picture of the processes operating during various stages of a global warming event. For example, at some stage photosynthetic plankton will draw down CO_2 from the atmosphere, producing organic matter that will be buried in rocks, and leading to global cooling that possibly heralds the end of the warming event[8,9]. In this regard, the event of 93 Myr ago can serve as a test run for the possibility of seeding the modern ocean with nutrients to promote photosynthesis and CO_2 reduction.

But a better understanding of that event and its relevance to modern global change requires knowledge of the scale of the volcanism (and so the rate and amount of CO_2 input), and how it is related to the OAE. These questions are only partially addressed by Turgeon and Creaser[1]. They show that there is a temporal relationship between the LIP and the OAE that implies a causal connection, but they offer no proof for the nature of that connection.

One possibility is that the large amounts of CO_2 may have seeded the ocean with micronutrients such as iron, stimulating plankton to produce large quantities of organic matter[11]. Oxidation of this organic matter would have stripped the ocean of oxygen, leading to the OAE (Fig. 1). Whether such a process alone could cause a global anoxic event has yet to be determined, but I remain doubtful that it could. Perhaps that effect was exacerbated by oceanic stratification (Fig. 1), a result of the warming produced by volcanic CO_2 that severely inhibited mixing in the ocean.

Especially significant is the time lag between the initial climatic perturbation, produced by the emission of huge quantities of CO_2 into the atmosphere, and its long-term consequences. Based on the rate at which the sedimentary rocks are known to have accumulated, Turgeon and Creaser[1] estimate that the volcanic pulse preceded the OAE by 23,000 years. Although the lag between trigger and catastrophe might be heartening to those concerned about the impacts of global warming, the time it takes signals to mix in the oceans today (about 1,500 years) implies that the lag time was shorter; other abrupt warming events in the geological record have similar, shorter lags. If the response time to the LIP volcanism was indeed longer, it suggests either that ocean circulation was extraordinarily sluggish before the event, or that there was a complicated connection between the trigger and the OAE. Determining how the volcanism of 93 Myr ago wreaked havoc on the warm Earth is another challenge for geologists endeavouring to understand the complex behaviour of our planet. ■

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SIGNAL TRANSDUCTION

Linking nutrients to growth

Vittoria Zinzalla and Michael N. Hall

How cells sense nutrients to control growth is largely unknown. One missing link involved in conveying the nutrient signal to the TOR protein, which regulates growth, seems to be the Rag proteins.

In mammalian cells, nutrients (such as amino acids), growth factors and cellular energy together trigger a molecular signalling pathway, mediated by the protein TOR, that controls cell growth. Deregulation of this pathway is implicated in cancer, and TOR inhibition by the anticancer drug rapamycin prevents unruly cell growth. Extensive research has led to characterization of many components of this signalling pathway. A remaining question is how amino acids activate TOR. Two teams (Kim *et al.*[1] reporting in *Nature Cell Biology* and Sancak *et al.*[2] writing in *Science*) now provide an important clue.

TOR ('target of rapamycin') is an evolutionarily highly conserved protein kinase that is found in two functionally and structurally distinct multiprotein complexes: TORC1 and TORC2 (ref. 3). TORC1 controls many cellular processes that ultimately determine cell growth, including protein synthesis, ribosome formation, nutrient transport and autophagy (a survival mechanism that kicks in in response to starvation).

Activation of TORC1 requires simultaneous availability of amino acids, growth factors and energy. Although inputs from growth factors (such as insulin) and energy are relatively well understood, determining the way that amino acids trigger TORC1 activation has been elusive. Amino-acid depletion results in rapid dephosphorylation of two molecules downstream of TORC1, S6K and 4E-BP, whereas addition of amino acids leads to rapid, TORC1-dependent phosphorylation of these molecules. But what is the molecular link between the amino-acid signal and TORC1 activation?

To answer this question, Kim *et al.* and Sancak *et al.* used complementary approaches. Using the technique of RNA interference (RNAi), Kim and colleagues[1] performed a screen with the S2 cell line of the fruitfly *Drosophila* to search for GTPase proteins that regulate S6K phosphorylation in response to amino

analysis in mammalian cells to identify new binding partners of TORC1. Both teams independently identified Rag GTPase proteins as mediators of the amino-acid signal to TORC1. Sancak and colleagues further showed that these regulatory molecules interact with raptor, a component of TORC1. Meanwhile, Kim *et al.* concentrated on the physiological aspects of the Rag GTPases' function, demonstrating their role in TORC1-mediated regulation of autophagy and cell size in *Drosophila*.

Regulatory GTPases come in different varieties and are commonly components of signalling pathways. These proteins are active when bound to the nucleotide GTP and inactive when bound to GDP — the product of GTP hydrolysis. The intrinsic enzymatic activity of the GTPases converts the GTP to GDP. Specific GTPase-activating proteins (GAPs) stimulate this intrinsic enzymatic activity of the GTPases, and another group of proteins called GTPase exchange factors (GEFs) mediate dissociation of GDP from GTPases so that the GTPases can bind to a new GTP molecule and resume activity. Thus, GAPs inhibit and GEFs stimulate the signalling function of GTPases.

There are four Rag proteins (RagA–D), with high sequence similarity existing between RagA and RagB, and between RagC and RagD. These proteins function as heterodimers — RagA or B binding to RagC or D (ref. 4). Rag heterodimers form independently of GTP/GDP binding status. Nonetheless, GTP binding to the RagA/B subunit is crucial for the activation and localization of the heterodimer. The two studies[1,2] show that a RagA/B that cannot bind to GTP also fails to stimulate TORC1, and a constitutively active RagA/B supports TORC1 activity even in the absence of amino acids.

But what is the consequence of Rag binding to TORC1? Sancak *et al.* propose that amino-acid-activated Rags are molecular matchmakers for TORC1 and another GTPase called Rheb. When bound to Rag, TORC1 is some-

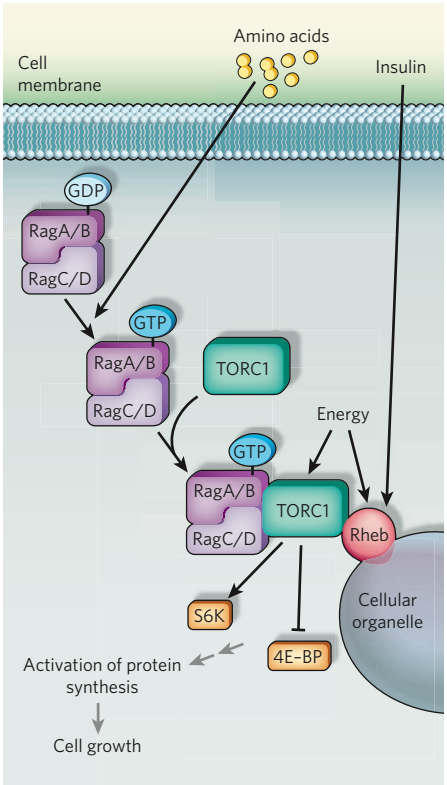

Figure 1 | Passing on the message. Amino acids are transported into the cell where they trigger a molecular signalling pathway that is mediated by the multiprotein complex TORC1. Two studies[1,2] show that Rag GTPases — heterodimers of RagA or RagB and RagC or RagD — are involved in TORC1 activation in response to this nutrient signal. By binding to TORC1, Rag GTPases mediate its transfer to intracellular membranes that contain another GTPase, Rheb. There, other signals such as cellular energy and growth factors (insulin) integrate with the amino-acid signal, leading to Rheb-mediated activation of TORC1 and phosphorylation of its downstream effectors S6K and 4E-BP, which ultimately lead to protein synthesis and cell growth.

the membrane of a so far ill-defined organelle. Rheb then directly stimulates the kinase activity of TORC1, which leads to S6K and 4E-BP phosphorylation. Amino acids impinge on this mechanism at the level of GTP loading of RagA/B (Fig. 1).

Previous work[5] has shown that the Rheb–TORC1 interaction depends on amino-acid availability, and that TORC1 inhibition after amino-acid withdrawal can be overcome through increased Rheb expression. Intriguingly, inactivation of the TSC1–TSC2 complex, a GAP that inhibits Rheb, cannot overcome the effect of amino-acid withdrawal[6], suggesting that amino acids probably mediate TORC1 interaction with activated Rheb, rather than activation (GTP loading) of Rheb. (Insulin and energy mediate the activation of Rheb through inhibition of TSC1–TSC2 GAP activity.)

The molecular mechanism by which Rheb activates TORC1 once Rheb has integrated amino acid, insulin and energy signals, and has