H3Q5his, and this co-localization correlated with the increased expression of genes in these locations.

The only brain region that is rich in histamine-releasing neurons is the hypothalamic tuberomammillary nucleus (TMN). The authors found that H3Q5his was enriched in this brain region. Because the TMN is involved in the circadian rhythm – the body's internal clock that controls when in a 24-hour period certain physiological functions occur – Zheng *et al.* next investigated whether neurons in the TMN show rhythmic gene expression, and whether this depends on H3Q5his.

Genomic analysis of mouse TMN tissue collected at regular intervals over a 24-hour period revealed rhythmic gene expression patterns, and striking fluctuations in levels of H3Q5his. The highest levels were detected when mice were awake and active, and the lowest when mice were inactive. Notably, these fluctuations were particularly prominent in clock genes, which encode proteins that control the circadian rhythm. Furthermore, when Zheng *et al.* treated active mice with a sedative to artificially induce the inactive phase, levels of H3Q5his, H3Q5ser and WDR5 were reduced across the genome and especially at a set of clock genes that are regulated by the transcription factors CLOCK and BMAL1.

Do monoaminylated H3Q5 marks have a causal role in circadian rhythm? Zheng *et al.* introduced a mutant version of histone H3 that lacks glutamine 5 and therefore cannot be modified with monoamine groups into TMN neurons of mice. Although rhythmic gene expression was seen in a large set of genes (including established circadian genes) in mice expressing normal H3, transcriptional rhythmicity was lost in the mutant samples. Finally, the authors examined animal behaviour: locomotor activity was assessed by measuring the distance that mice travelled in their cages throughout the day. Mice with mutant H3 showed impaired locomotor activity during the day and a considerable locomotory deficit in transitions between active and inactive phases.

Zheng and colleagues shed light on a complex histaminylation-dependent mechanism through which the TMN controls circadian rhythms (Fig. 1). Several questions remain for future research. Do glutamine sites other than H3Q5 undergo histaminylation? What is the effect of neighbouring epigenetic marks, other than H3K4me, on histaminylation and vice versa? How does H3Q5his affect binding and activities of the extensive family of writers, erasers and readers that are specific to H3K4me?

When intracellular concentrations of monoamines are insufficient for monoaminylation, TG2 was found to catalyse the conversion of histaminylated glutamine (H3Q5his) to a glutamate residue (H3E5). This conversion is drastic because the charge at this position flips from positive (histaminylated glutamine) to negative (glutamate), and H3E5 can no longer be modified with monoamines. Therefore, the physiological and disease outcomes associated with this phenomenon need to be explored.

The most challenging direction for research in the future will be to characterize how H3Q5his and H3Q5ser are linked to antidepressant medications[6] and sensory-processing behaviours[7], and how dopaminylated H3Q5 is involved in addiction and behavioural adaptability[3]. Addressing these issues will broaden scientists' understanding of the link between dynamic epigenetic processes in the brain and the pathways that dictate behaviour.

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Structural biology

How a nitrogen-fixing enzyme avoids oxygen

Amy C. Rosenzweig

The enzyme nitrogenase 'fixes' nitrogen gas to form bioavailable ammonia, a vital process for life. Two studies solve a long-standing mystery: how a small protein saves nitrogenase from destruction by oxygen. **See p.991 & p.998**

The most abundant form of nitrogen – gaseous dinitrogen (N_2) – constitutes 78% of the atmosphere, but cannot be used directly by organisms for metabolism. Instead, dinitrogen must be converted into ammonia (NH_3) or bioavailable ammonium (NH_4^+, the salt form of ammonia used in fertilizers) through a challenging process known as fixation. In nature, bacteria called diazotrophs fix nitrogen using nitrogenase enzymes[1,2]. Isolated nitrogenases are destroyed by oxygen gas in minutes, but in diazotrophs they are shielded from oxygen, in part, by a small protein called FeSII (also referred to as Shethna protein II after its discoverer[3]). Writing in *Nature*, Franke *et al.*[4] (page 998) and Narehood *et al.*[5] (page 991) unveil the long-awaited molecular details of how FeSII exerts its protective effect.

Why do we need to understand this protection mechanism? Some diazotrophs live in leguminous plants, providing them with fixed nitrogen, but most crops need fertilizer produced industrially through the energy-intensive Haber–Bosch process to grow well. This requirement would be circumvented if crops or crop-associated bacteria could be engineered to express nitrogenases[6]. However, such nitrogenases would have to function in soil environments that contain oxygen, making it crucial to understand how the enzymes could be protected from this gas.

Nitrogenases are large and complex enzymes, befitting the difficulty of the fixation process. They consist of two components: an iron (Fe) protein and a molybdenum–iron (MoFe) protein, which must come together and then separate eight times to catalyse the conversion of one dinitrogen molecule into two ammonia molecules[1,2]. During this process, eight electrons are transferred successively from a cluster of iron and sulfur atoms (the [4Fe:4S] cluster) in the Fe protein to another cluster of these atoms (the [8Fe:7S] cluster, also known as the P cluster) in the MoFe protein. From there, the electrons are passed to the FeMo cofactor – the site in the MoFe protein at which dinitrogen is fixed.

Because both the Fe and MoFe proteins are inactivated by oxygen, all biochemical studies of nitrogenase must be executed under strictly anaerobic (oxygen-free) conditions. However, an early study[7] showed that nitrogenase in diazotrophs resists oxygen damage because FeSII forms a protective complex with the Fe and MoFe proteins – a striking finding, given that FeSII is about one-tenth the size of the complex formed by the Fe and MoFe proteins alone. The Fe and MoFe proteins are unreactive when in the protective complex, but once the oxygen threat is gone, FeSII dissociates, and the other two proteins reactivate so that fixing can resume. How FeSII protects nitrogenase from oxygen damage has remained a mystery for more than 50 years.

The advent of cryogenic electron microscopy (cryo-EM), along with innovations for the anaerobic preparation of cryo-EM samples, has allowed the structure of nitrogenase to be visualized as it catalyses fixation[8]. Building on these successes, Narehood *et al.* and Franke *et al.* prepared samples of nitrogenase during catalysis and exposed them to oxygen for several minutes to promote the formation of the protective complex, which they then trapped for cryo-EM analysis.

The authors observed unusual V-shaped particles in their samples, some of which formed extended helical filaments. Each V-shaped particle consists of two copies of the Fe and MoFe proteins, glued together by a single FeSII protein (Fig. 1). The previous cryo-EM study[8] showed that, during catalysis, one or two Fe proteins dock with the MoFe protein. The current studies reveal that the FeSII protein jams itself between two MoFe proteins and two Fe proteins, not only creating the V shape, but also facilitating polymerization of the proteins into a continuous filament.

How does this V-shaped complex protect nitrogenase from oxygen? By driving a wedge between the Fe and MoFe proteins, FeSII separates the surfaces of those proteins that interact during catalysis. One end of FeSII completely covers the Fe protein's [4Fe:4S] cluster, which is usually exposed and is the most oxygen-sensitive cluster in nitrogenase. Under catalytic conditions, this cluster sits about 15 ångströms away from the P cluster in the MoFe protein, enabling electron transfer[8]. By contrast, binding of FeSII in the protective complex holds the clusters more than 30 Å apart – too far for electron transfer. Other parts of FeSII block access of oxygen to the FeMo cofactor.

The V-shaped structure also explains why the Fe protein is protected from oxygen only in the presence of the MoFe protein[9]: although FeSII binds first to the Fe protein, the MoFe protein is needed to form a stable protective complex and to 'seed' the formation of filaments. Therefore, the small but mighty FeSII defends the nitrogenase against oxygen by pulling the enzyme's components into an inactive assembly.

FeSII has its own cluster of iron and sulfur atoms, the [2Fe:2S] cluster, which can exist in oxidized and reduced states. Franke *et al.* report the structure of FeSII in the reduced state. The authors observe that two loops of the protein – one known as the N-loop, and another that houses the cysteine amino-acid residues that bind to the [2Fe:2S] cluster – are tightly folded back on FeSII, precluding their interaction with the nitrogenase proteins. By contrast, when the [2Fe:2S] cluster is oxidized, the N-loop adopts several conformations[9,10] that enable it to dock first with the Fe protein and then with the MoFe protein.

The reduction potential of the FeSII [2Fe:2S] cluster – a measure of the cluster's tendency

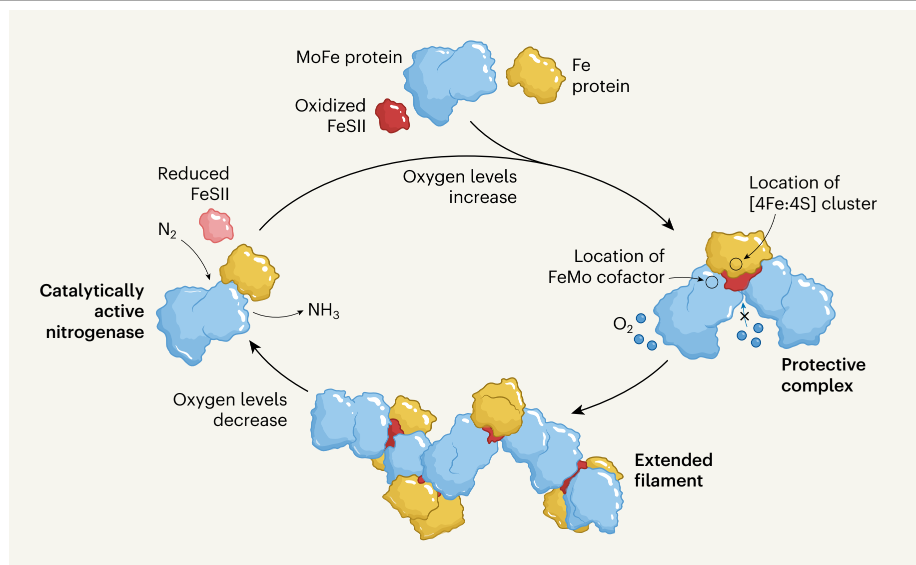

Figure 1 | The FeSII protein protects nitrogenase from oxygen. The nitrogenase enzyme catalyses the conversion of nitrogen gas (N_2) to bioavailable ammonia (NH_3), and consists of two components: the Fe protein and the MoFe protein. Two papers[4,5] report how a third protein, FeSII, protects nitrogenase from oxygen. When oxygen levels are low, FeSII is in a reduced form that does not interact with nitrogenase. But when oxygen levels are high, FeSII becomes oxidized and changes conformation. It then forms a V-shaped complex with two molecules each of the Fe and MoFe proteins. In this complex, FeSII blocks access to the Fe protein's [4Fe:4S] cluster and to the FeMo cofactor in the MoFe protein, thereby protecting nitrogenase from oxygen. FeSII also wedges apart the Fe and MoFe proteins, preventing interactions that would enable catalysis. The V-shaped particles further assemble into extended filaments. When oxygen levels decrease, the filaments disassemble by an unknown mechanism and nitrogenase catalysis resumes.

to be reduced – therefore defines a trigger point for nitrogenase protection. As the environment in the cell becomes more oxidizing than the reduction potential of the [2Fe:2S] cluster (−262 millivolts)[11], FeSII switches to its oxidized conformation and can bind to nitrogenase, which becomes deactivated. Then, when oxygen levels fall, FeSII becomes reduced and dissociates from the protective complex, reactivating the enzyme (Fig. 1).

Reduction of FeSII and disassembly of the protective complex are easily accomplished *in vitro* using a chemical reductant, but how FeSII is reduced in cells remains an open question. One conundrum is that, in the process of preventing electron transfer from the Fe protein's [4Fe:4S] cluster to the MoFe protein's P cluster, FeSII positions its own [2Fe:2S] cluster within 12 Å of the [4Fe:4S] cluster. This arrangement would facilitate reduction of the oxidized [2Fe:2S] cluster by the reduced [4Fe:4S] cluster. Franke *et al.* suggest that such reduction further locks FeSII into place, ready for MoFe-protein binding and filament formation. However, in this scenario, it is unclear how dissociation of FeSII from the protective complex would occur when oxygen levels fall, because FeSII would already have been reduced.

Narehood *et al.* alternatively suggest that the Fe protein would already be oxidized under conditions that promote protection by FeSII. They demonstrate that reduction of oxidized FeSII by reduced Fe protein can indeed occur *in vitro*, but they also show that the protective complex forms when all of the components are oxidized – suggesting that electron transfer between the Fe protein and FeSII is not a necessary step in the protective mechanism. The authors propose that other proteins, such as ferredoxins or flavodoxins, transfer electrons to FeSII, thereby reducing it and causing it to dissociate from the protective complex. Exactly how nitrogenase protection is reversed in the cell therefore remains to be elucidated.

An understanding of the remarkable mechanism by which this small protein, FeSII, protects the much larger nitrogenase by shifting the latter's shape might one day enable researchers to engineer plants to produce their own fertilizer. More broadly, Franke *et al.* and Narehood *et al.* have revealed a new model for enzyme regulation through protein–protein interactions.

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Space physics

Chirping chorus rings out from outer space

Richard B. Horne

Bursts of electromagnetic radiation that share similarities with birdsong have long been observed close to Earth. A detection farther out in space confirms a key part of the theory of their origin, but also poses questions. **See p.813**

Many readers will be familiar with the dawn chorus, the birdsong that can be heard at the break of day, especially in spring. But this performance lends its name to another type of chorus – one of intense electromagnetic bursts – chiefly because, when these waves are converted into audio signals, they sound similar to birds chirping. These 'chorus waves' have been observed by radio receivers at the Halley Research Station in Antarctica since the late 1960s. They originate in space, high above a planet's equator, and they loosely follow the path of its magnetic field. Earth, Jupiter and Saturn are all known to host chorus waves, but now, on page 813, Liu *et al.*[1] report the discovery of chorus waves in an unlikely location, some 160,000 kilometres away from Earth.

Chorus waves last only a few tenths of a second and have a characteristic rising frequency[2], which gives them their chirping sound. Each burst is slightly different, but the signals often repeat for hours. They are usually detected in areas of space where Earth's magnetic field resembles that of a giant bar magnet (a dipole), out to around 51,000 km above Earth's surface, beyond geostationary orbit, where a satellite's orbit matches the rotation of Earth. However, Liu *et al.* found evidence of chorus waves in a part of space in which Earth's magnetic field is highly distorted (Fig. 1). Their observations are important because the dipolar nature of the magnetic field has a crucial role in how chorus waves are generated – or thought to be generated. The discovery doesn't rule out the existing theory, because the expected magnetic field gradients could still be present, but it means that scientists need to take a closer look.

The generally accepted theory is that chorus waves are created by a phenomenon called a plasma instability, which occurs when an ionized gas (a plasma) strays from its equilibrium state. In the case of chorus waves, it involves an unstable population of electrons spiralling in a planet's magnetic field. These electrons emit radiation as they spin, and this radiation is amplified by wave–particle interactions with other electrons to form chorus waves. Liu *et al.* showed that the plasma is unstable, and that energy is transferred from electrons to electromagnetic waves. They also observed long-sought-after evidence to support the theory – the observation of an 'electron hole', a conspicuous absence of electrons.

The theory is a nonlinear one, and it goes like this. The energy transfer is most efficient when there is 'resonance' between the waves and the electrons, meaning their frequencies are matched. This can happen because the electric and magnetic fields of the radiation in chorus waves rotate mainly in a clockwise direction and the electrons spiral around Earth's magnetic field in the same direction. If the waves and electrons are travelling in opposite directions, the frequency of the waves is shifted to the frequency at which the electrons spiral, which is known as the cyclotron frequency.

As the waves grow in energy, they react back on the electrons, ordering their motion into bunches that become trapped at a particular point of the waveform with respect to the magnetic field generated by the chorus waves (their phase). In effect, the electrons spiral around the planet's magnetic field in unison with that of the chorus waves, but the phases are fixed with respect to each other; the electrons become ordered into a spiral current.

Theory suggests that such ordering will lead to a dearth of particles at other parts of the cycle[3]. This is what's known as an electron hole,

Figure 1 | Chorus waves in outer space. Chorus waves are bursts of electromagnetic radiation that have been observed for decades in regions of space where Earth's magnetic field resembles that of a bar magnet. **a**, Liu *et al.*[1] detected chorus waves in a part of space where this field is highly distorted, an observation that has implications for understanding how chorus waves are generated. **b**, Chorus waves are thought to form when the radiation emitted by electrons spiralling in Earth's magnetic field is amplified by interactions with other electrons. The electrons spiral in the same direction as the rotation of the electric (not shown) and magnetic fields of the radiation waves, so if the waves and electrons are travelling in opposite directions, the wave frequency shifts to that of the electrons' motion. This causes the electrons to order into bunches, leaving an 'electron hole' in some regions, which is exactly what Liu *et al.* observed. The challenge now is to understand why it was observed so far from Earth.