viral RNA, and Batra and colleagues transferred DNA sequences encoding these proteins into human cells grown *in vitro*. They found that the expression of VP30, at both the RNA and protein level, depended on the level of RBBP6, with high levels of RBBP6 being associated with low levels of VP30 expression and with low activity of the viral polymerase complex. When the authors used a technique called gene silencing to reduce the expression of RBBP6 in the human cells expressing Ebola proteins, the activity of the Ebola viral polymerase complex and viral replication was increased.

The authors carried out an X-ray structural analysis to model the interaction between VP30 and RBBP6. These structural results, and those from their other experiments investigating protein binding to VP30, revealed that a key feature of interest in RBBP6 is a sequence of 23 amino-acid residues (a peptide) that includes a motif called PPxPxY (in which P is the amino acid proline, Y is the amino acid tyrosine and x is any other amino-acid residue). This motif inserts into a cleft in VP30 that is known[8] to bind to an Ebola protein called NP (Fig. 1). NP is part of the viral polymerase complex and serves as a scaffold that binds to viral RNA. VP30 binding to NP promotes viral RNA synthesis. The researchers noted that the PPxPxY motif is also present in NP in a region that binds to VP30. This suggests that NP and RBBP6 might compete for binding to VP30.

In *in vitro* experiments, Batra *et al.* showed that the binding between RBBP6 and VP30 was five times stronger than that between NP and VP30, indicating that RBBP6 should have the capacity to effectively sequester VP30 from NP. Their experiments suggest that this peptide motif alone has a key role in limiting the interaction between VP30 and NP. Furthermore, if, instead of RBBP6, an engineered chimaeric protein composed of a PPxPxY-containing peptide and a fluorescent marker called green fluorescent protein was expressed in human cells expressing Ebola proteins, the chimaeric protein decreased Ebola polymerase complex activity and viral propagation compared with the effect observed in cells that received only green fluorescent protein.

Because Batra and colleagues' approach to identifying the interactions between viral and host proteins is based on the expression of single types of viral protein, it does not identify host-protein interactions that might occur only when viral proteins are part of multi-protein complexes. Further analyses will therefore be needed to identify any such interactions, and to verify that the interactome map is correct. Also, because these experiments were conducted using genetic-engineering techniques, rather than studying a natural process of viral infection of human cells, it is difficult to assess the extent to which these events ultimately affect the level of viral replication. It is possible that, like other host antiviral proteins, RBBP6 levels vary and are subject to regulation by unknown mechanisms. Further study is needed to understand exactly how RBBP6 affects Ebola virus replication.

During the 2013–16 Ebola outbreak[9,10], no mutations in VP30 were reported in the region of the protein that corresponds to the RBBP6 binding site. This suggests that evolutionary selective pressure to evade host targeting by RBBP6 is limited, or that a viral mutation that drives resistance to RBBP6 is not selected for because it has a detrimental effect on the virus — possibly because such a mutation might also affect an interaction between VP30 and NP that is essential for viral replication. It would be interesting to test whether bats inhibit Ebola virus replication using RBBP6.

A key discovery of this study is that a peptide that includes the PPxPxY motif, when fused to green fluorescent protein, is sufficient to inhibit virus replication *in vitro* (the effect *in vivo* was not evaluated). The cleft on VP30 to which this peptide binds could therefore be a promising target for efforts to develop antivirals against Ebola. Although it is not known whether the peptide inhibits viral replication when it is not fused to green fluorescent protein, this discovery could provide a starting point for developing and optimizing other small compounds that inhibit viral replication. That might lead to the development of a class of antiviral that has high specificity for the Ebola virus. ■

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OCEAN BIOGEOCHEMISTRY

A diagnosis for marine nitrogen fixation

Nitrogen gas dissolved in the ocean must be fixed — converted into more-reactive compounds — before it can be used to support life, but the regions in which this nitrogen fixation occurs have been elusive. Not any more. SEE ARTICLE P.205

NICOLAS GRUBER

In 1934, the oceanographer Alfred C. Redfield took a leap into the unknown. He posited[1] that marine nitrogen-fixing organisms — microorganisms that convert nitrogen gas (N_2) dissolved in the sea into 'fixed' nitrogen compounds that can be used by other organisms to sustain life — might help to explain why the ratio of fixed nitrogen (N) and phosphorus (P) in sea water is similar to their ratio in marine phytoplankton, even though no nitrogen-fixing organisms had been identified at the time. Since then, the search for the regions in which nitrogen fixation occurs, and the role of fixation in controlling the marine nitrogen cycle, have engaged biogeochemists and biologists alike[2]. But despite much progress, the patterns, rates and limiting factors that control marine nitrogen fixation have remained elusive[3].

In 2007, a study[4] that used a geochemical method called the P* approach suggested that a large fraction of marine nitrogen fixation occurs in the eastern tropical Pacific Ocean. This is a region in which depleted oxygen levels in the water column cause fixed nitrogen to be converted back to N_2, a process called denitrification. The P* approach analyses the relative abundance of nitrate (the major form of fixed nitrogen) and phosphate in sea water, and estimates the transport and mixing of these nutrients using an ocean-circulation model. In contrast to the results of the P* method, direct measurements of nitrogen-fixation rates suggest that there is a clear spatial separation between nitrogen fixation and denitrification[5–7]. On page 205, Wang *et al.*[8] suggest a way of reconciling the results of these two approaches.

The authors pick up many of the threads initially laid out by Redfield and also used in the P* approach. Thus, they use a diagnostic model to work out the sources and sinks of fixed nitrogen and phosphorus implied by the transport and mixing of nitrate and phosphate in sea water, and compare this with the N:P ratio in marine phytoplankton

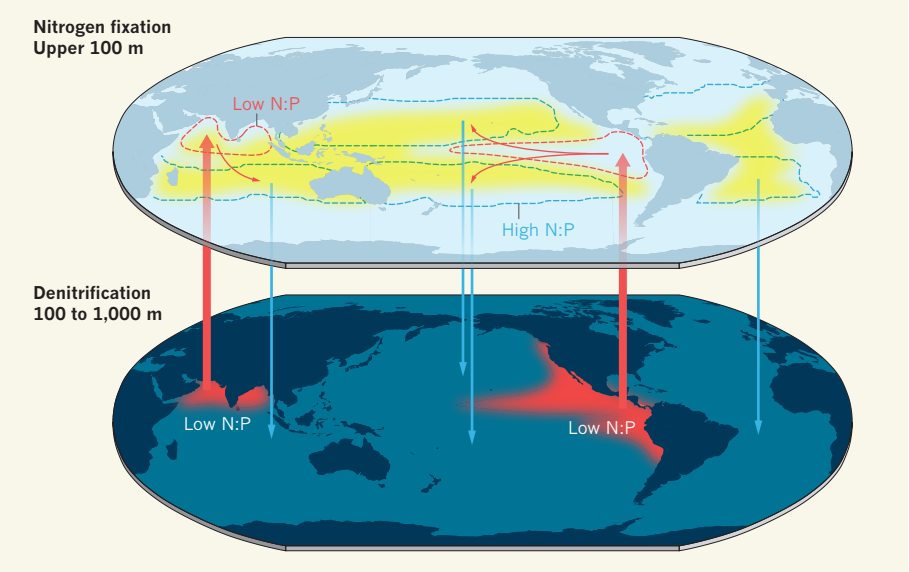

Figure 1 | Links between marine nitrogen fixation and denitrification. Nitrogen gas dissolved in the sea is 'fixed' by microorganisms in the upper 100 metres to form nitrogen compounds that are used by other organisms to sustain life. The reverse process — denitrification — occurs at depths of 100–1,000 m in the Indian Ocean and the eastern tropical Pacific Ocean (red areas). Nutrients in waters upwelling from the denitrification zones have very low ratios of nitrogen to phosphorus (N:P). A geochemical method known as the P* approach inferred that most nitrogen fixation occurs close to these denitrification zones[4], but Wang and colleagues' analysis[8] now suggests that it occurs mainly in the subtropical gyres (yellow areas) downstream of the tropical upwelling regions. The high levels of nitrogen fixation in the gyres contribute to the formation of biomass that has high N:P ratios, which sinks to the ocean's interior (blue arrows) and compensates for the loss of nitrogen caused by denitrification.

and in the biomass that is exported from the surface to the deep ocean; they assume that any mismatch must be the result of nitrogen fixation or denitrification. But, in contrast to the P* study, Wang and colleagues' analysis incorporates an observationally constrained model of the ocean's annual mean circulation[9], uses nutrient data from throughout the water column rather than just from the surface, and takes into account the variation of N:P ratios in marine phytoplankton in different regions, rather than assuming a constant ratio. They complement their approach by using a model of ocean biogeochemistry and ecology to investigate the ecological controls on marine nitrogen fixation.

The authors' diagnostic model infers that most marine nitrogen fixation occurs in the ocean's subtropical gyres — vast circulation systems that span entire ocean basins at mid-latitudes, and which have low levels of nutrients (Fig. 1). In these regions, nitrogen fixation provides more than 30% of the nitrogen required to fuel the production of biomass and its subsequent export to the deep ocean. The model suggests that, by contrast, very low levels of nitrogen fixation occur in the eastern tropical Pacific and the Arabian Sea, the other tropical regions in which water column denitrification is prevalent.

The shift of nitrogen fixation away from denitrification zones to the subtropical gyres produces a geographical pattern that is much more in line with directly measured fixation rates[5] than with the pattern inferred by the P* approach. In particular, the pattern from the diagnostic model is a better match for the low rates measured in the eastern tropical Pacific[6] (where the P* approach inferred that fixation rates are highest[4]), and the high rates observed in the western Pacific[7].

The authors argue that the shift is mostly the result of their analysis accounting for the variable N:P ratios in phytoplankton and exported biomass[4]. Indeed, Wang *et al.* infer that the N:P ratio of exported biomass varies substantially, from values as high as 21 in the subtropical gyres to as low as 17 in the tropical upwelling regions. Crucially, the authors show that this ratio varies spatially in a similar pattern to that for the ratio of nitrate to phosphate dissolved in sea water. This means that — in contrast to the P* method — their geochemical diagnosis decouples nitrogen fixation from the regions that have low N:P ratios associated with the upwelling of waters that had been subject to denitrification (Fig. 1).

Wang *et al.* estimate that marine nitrogen fixation adds about 160 (130–220) teragrams of nitrogen per year to the global ocean (1 Tg is 10^{12} grams). Together with the inputs from rivers and atmospheric sources, about 200 Tg of nitrogen is added to the ocean annually. This is countered by a diagnosed denitrification (a loss) of approximately the same amount. The authors therefore conclude that the global nitrogen budget is almost balanced, and involves input and loss rates that are in line with many previous estimates of the global nitrogen budget[10].

The simulations obtained from Wang and co-workers' biogeochemistry–ecology model suggest that a complex, global mosaic of limitations acts on marine nitrogen fixers. Fixation across most of the Atlantic is limited by the availability of phosphorus, whereas iron is the limiting factor in most of the eastern Pacific. In contrast to the findings of the P* approach[4], the effect of the N:P ratio of nutrients seems to be small. Unexpectedly, Wang and co-workers also find that zooplankton strongly affect the abundance and activity of nitrogen fixers: in a simulation in which grazing of nitrogen fixers by zooplankton was strongly curtailed, nitrogen fixation shifted back from the gyres to the eastern tropical Pacific.

The insights gained from the new study are a major step forward in our understanding of the marine nitrogen cycle. In particular, the much larger spatial separation of nitrogen fixation and denitrification compared with the results of the P* approach, and the less-important role of the N:P ratio of nutrients in controlling marine nitrogen fixation, imply that the negative feedback between nitrogen fixation and denitrification is weaker than had been thought. This feedback is key to maintaining the balance between sources and sinks of fixed nitrogen in the ocean[10] — a weaker feedback could lead to stronger fluctuations of the fixed-nitrogen inventory in the sea.

Wang and co-workers' study has some limitations, however. For example, their results could be biased because their models don't account for seasonal and year-to-year variability — the latter causes large variations in denitrification in the eastern tropical Pacific[11]. The authors also only partially consider the impact of the substantial perturbation of the marine nitrogen cycle that is associated with increased inputs of fixed nitrogen from the atmosphere and rivers during the Anthropocene (roughly the past two centuries), which has already led to measurable changes in the N:P-nutrient ratios in the ocean[12]. Modelling studies[13] suggest that this perturbation has led to substantial changes in marine nitrogen fixation and denitrification, and to an unbalanced nitrogen budget. Finally, the simulations from the authors' biogeochemistry–ecology model consider essentially just one type of nitrogen fixer, whereas a diverse range of organisms are capable of nitrogen fixation[14]. The activity of each group of organisms is probably controlled by different sets of processes.

Nevertheless, Wang *et al.* have provided strong evidence that nitrogen fixers in the ocean have a well-defined niche in the warm waters of the subtropical gyres — which are generally nutrient-poor, but have enough iron and phosphate to allow nitrogen fixers to compete with other phytoplankton. The new work thus resolves a major discrepancy that has occupied researchers over the past decade[3]. I am confident, however, that the marine nitrogen cycle has not yet revealed all of its secrets: the areas in which nitrogen fixation occurs

might no longer be elusive, but the nitrogen cycle remains a treasure trove for discovery. ■

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COMPUTATIONAL DRUG DISCOVERY

Bigger is better in virtual drug screens

A system has been devised that computationally screens hundreds of millions of drug candidates — all of which can be made on demand — against biological targets. This could help to make drug discovery more efficient. SEE ARTICLE P.224

DAVID E. GLORIAM

Screening for effective drugs is tremendously expensive and inefficient. High-throughput screens can cover up to a few million compounds, but this is just a minute fraction of the total number (10^{63}) of 'drug-like' molecular structures thought to exist[1,2]. Moreover, typically, less than 0.5% of compounds tested in screens turn out to have activity at the chosen biological target[3]. There is therefore much interest in expanding the number of molecules that can be explored in the early screening stages of drug-discovery programmes, while limiting the number that need to be synthesized and assayed in the laboratory. On page 224, Lyu *et al.*[4] achieve both these goals by computationally screening ultra-large compound libraries to prioritize compounds to be synthesized and assayed.

Physical drug-screening libraries are predominantly limited to compounds that are available in-house or off-the-shelf from commercial catalogues. By contrast, Lyu *et al.* docked — computationally simulated the binding of — 170 million compounds that could be made on demand by a commercial supplier. More than 97% of these compounds were not available from other vendors' collections. The number of compounds in the authors' make-on-demand library has since grown, and is projected to contain 1 billion within 2 years. The authors have made this library available as a public database of 3D molecular structures (see go.nature.com/2sywxlt), which can be used by any researchers for virtual screening.

To evaluate how well virtual screening works with this extremely high number of chemical structures, Lyu and colleagues first investigated whether a few tens to hundreds of known ligand molecules could be distinguished within the full library of 170 million members using docking scores — which quantify how strongly compounds bind to given biological targets. The authors virtually screened the libraries against two targets: the enzyme AmpC β-lactamase and the D_4 dopamine receptor. The top-scoring molecules did indeed include known ligands for these targets and their close structural analogues.

The authors went on to synthesize the top-scoring compounds that had not previously been identified as ligands, as well as some analogues of these compounds. Many of these were found to be pharmacologically active in assays. Impressively, one of the compounds is the most potent AmpC inhibitor known among those that do not bind irreversibly to the enzyme (potency describes the biological response of a target to its ligand, rather than the binding affinity of the ligand for the target).

One of the D_4-stimulating compounds has unprecedented affinity for D_4 and selectivity for it over the related D_2 and D_3 receptor subtypes. Moreover, some of the other identified D_4 ligands were functionally selective — they preferentially activated either the G_i protein or the β-arrestin cellular signalling pathways

50 Years Ago

Test tube babies may not be just round the corner, but the day when all the knowledge necessary to produce them will be available may have been brought a stage nearer by the work reported by Dr R. G. Edwards and his colleagues this week … They have fertilized human egg cells *in vitro*, overcoming the problem of sperm capacitation — how to obtain sperm that are in the right state for fertilization — by using a medium similar to that recently used successfully with hamster sperm … Now that human oocytes can be fertilized *in vitro*, the obvious next step is to culture them to the blastocyst stage, as has already been achieved with the mouse and is likely soon with the rabbit.
From *Nature* 15 February 1969

100 Years Ago

While in London and examining the German guns in the Mall, I came across one with a burst shell in its breech … The shell seems to have burst while being loaded into the gun, and, although it is well opened out, only a small portion is missing. The retained pieces are of interest, for on their inner surfaces they are covered with a large number of small patches of very fine ripple marks. These must have been produced under the intense pressure of the explosion, for it is well known that the insides of shells are turned smooth, polished, and varnished. It is, of course, difficult to say whether a study of these ripple marks will prove of scientific value, but seeing that the gun and its shell are probably exposed to the rain, and as these unique ripple marks may soon corrode away, I … suggest that this particular gun and its shell should be protected against further injury by being removed to a geological museum.
From *Nature* 13 February 1919