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LETTER

The origins of the Redfield nitrogen-to-phosphorus ratio are in a homoeostatic protein-to-rRNA ratio

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Abstract

One of the most intriguing patterns in the biosphere is the similarity of the atomic nitrogen-to-phosphorus ratio (N:P) = 16 found in waters throughout the deep ocean and in the plankton in the upper ocean. Although A.C. Redfield proposed in 1934 that the intracellular properties of plankton were central to this pattern, no theoretical significance for N:P = 16 in cells had been found. Here, we use theoretical modelling and a compilation of literature data for prokaryotic and eukaryotic microbes to show that the balance between two fundamental processes, protein and rRNA synthesis, results in a stable biochemical attractor that homoeostatically produces a given protein:rRNA ratio. Furthermore, when biochemical constants and reasonable kinetic parameters for protein synthesis and ribosome production under nutrient-replete conditions are applied in the model, it predicts a stable protein:rRNA ratio of 3 ± 0.7, which corresponds to N:P = 16 ± 3. The model also predicts that N-limitation, by constraining protein synthesis rates, will result in N:P ratios below the Redfield value while P-limitation, by constraining RNA production rates, will produce ratios above the Redfield value. Hence, one of most biogeochemically significant patterns on Earth is inherently rooted in the fundamental structure of life.

Keywords

Microbes, N/P, nitrogen, phosphorus, plankton, polymerase, protein synthesis, Redfield ratios, ribosomes, rRNA.

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INTRODUCTION

The biochemistry of life establishes rigid proportions of chemical elements in key molecules: exactly one P atom is in each DNA and RNA nucleotide [but see Wolfe-Simon *et al.* (2009)], and each type of amino acid and nucleotide has a specific number of N atoms. The high biological demand for these two chemical elements manifests itself globally – N and P largely limit the world's primary productivity (Tyrrell 1999; Elser *et al.* 2007). But do the fixed proportions of these elements at the molecular scale themselves have a global imprint? The oceanographer A.C. Redfield himself suspected as much, writing: 'the relative proportion of phosphate and nitrate must tend to approach that characteristic of protoplasm in general' and invoking nitrogen fixation and denitrification as mechanisms that can maintain the pattern (Redfield 1934). A quarter-century later, Redfield developed this idea further into his seminal theory that the life in the sea controls its environment (Redfield 1958), an insight that considerably preceded the development of now well-known Gaia Hypothesis (Lovelock 1979; Falkowski 2000).

In essence, Redfield's N:P pattern involves two questions: (1) why is the N:P ratio 15–16 and not some other biologically plausible number? (2) How is the pattern maintained so robustly across large spatial and temporal scales? Redfield's original reasoning has been refined and quantified in several theoretical models that are geared towards answering the second question (Tyrrell 1999; Lenton & Watson 2000; Karl 2002; Lenton & Klausmeier 2007). These models, however, assume an intrinsic significance of the canonical Redfield ratio for plankton by explicitly introducing N:P of 16 into the models, where it can act as a 'stoichiometric magnet' to maintain the pattern in the ocean. This still leaves the first question open, which ultimately must be couched within deeper constraints imposed on organismal N:P by the basic stoichiometry of major cellular N and P pools (Geider & La Roche 2002; Klausmeier *et al.* 2004) or, as Redfield called them, by 'characteristics of protoplasm' (Redfield 1934, 1958).

We are now gaining a better understanding of the relative contributions of various macromolecules such as DNA, ATP, phospholipids, chlorophyll, free amino acids, surface-adsorbed nutrients and vacuoles to the overall cellular N:P (Geider & La Roche 2002; Sterner & Elser 2002; Sanudo-Wilhelmy *et al.* 2004). Most significantly, it has been shown that the largest contributors to cellular N:P ratios in most living things are proteins and RNAs (Falkowski 2000; Geider & La Roche 2002; Sterner & Elser 2002; Elser *et al.* 2003; Klausmeier *et al.* 2004). In most situations relevant to microbes, the single largest investment of N is into proteins, whereas the single largest investment of P is into ribosomal RNAs (rRNAs) (Geider & La Roche 2002; Sterner & Elser 2002). While the N:P ratio of the total protein and RNA in a cell is not necessarily precisely the same as the overall cellular N:P due to the relative contributions of other N and P pools, the central determinants of cellular N:P are nevertheless proteins and rRNAs.

For this reason Falkowski asked (Falkowski 2000): 'when cells are growing at an optimal rate, does the ratio of rRNA to protein conform to a common value for all prokaryotes and eukaryotes?' It is important to understand why the question centres on an 'optimal' growth, although the conditions in the ocean often are far from the optimal. Based on extensive analyses of the macromolecular composition of microbes grown under various conditions, it is known that protein:RNA can depend on both the growth rate and the identity of the

limiting nutrient (Leick 1968; Alroy & Tannenbaum 1973; Rhee 1978; Ross & Orlowski 1982). Hence, the emphasis on optimal conditions filters out local and temporally dynamic environmental effects and allows focus on the intrinsic biochemical properties and constituents of a cell – is there, deep in the very basic organization of life, an inherently significant N:P value?

Over the last decade, progress in answering this question has been made with the help of theoretical models that incorporate 'the growth rate hypothesis' (Elser *et al.* 1996; Sterner & Elser 2002), which identifies P-rich rRNAs as the main contributor to overall cellular P content due to the growth dependence of ribosome-driven protein synthesis. These analyses have found that the optimality of protein:RNA ratios depends on the environmental conditions, but no intrinsic significance for any specific protein:rRNA ratio or for an N:P ratio near the Redfield value has been found (Klausmeier *et al.* 2004, 2008; Karpinets *et al.* 2006). These recent theoretical results, along with observations that individual plankton species exhibit considerable variation in N:P, which can also depend on the environment, gave rise to the idea that an N:P ratio of 16 has no intrinsic significance (Klausmeier *et al.* 2004, 2008; Arrigo 2005; Mills & Arrigo 2010; Weber & Deutsch 2010). Instead, these models attempt to explain an N:P ratio of 16 as the result of the mixture of either (1) environmental conditions [nutrient-replete conditions benefit plankton with N:P = 8, while light, N- and P-limited conditions benefit plankton with N:P = 36–45 (Klausmeier *et al.* 2004)] or (2) taxonomically fixed N:P ratios, which do not depend on the environment [N:P for diatoms fixed at 11 and for all other plankton at 20 (Weber & Deutsch 2010)]. Such approaches suggest that, given a different mixture, a different value, say N:P = 12 or 25, can be seen biologically as plausible as 16. Such a reasoning not only undermines the validity of Redfield's original argument and the biogeochemical models that assign significance to N:P = 16 but also raises the possibility that significant shifts in marine N:P ratio could have happened in the past and can happen in the future (Broecker & Henderson 1998; Falkowski 2000; Pahlow & Riebesell 2000; Falkowski & Davis 2004; Lenton & Klausmeier 2007).

Here, we revisit Falkowski's question both theoretically and empirically. In the theoretical part, we model both rRNA and protein synthesis – the biomolecular processes that are deeply shared among all organisms regardless of any specifics of the energy acquisition pathway (e.g. photoautotrophs vs. organoheterotrophs). The novel part is in considering rRNA synthesis. Prior analyses have relied on 'the growth rate hypothesis' that schematically works in the direction: P → rRNAs → protein synthesis. Here, we incorporate the fact that rRNAs need to be synthesized as well, i.e. we consider schematically the opposite direction: N → proteins → rRNA synthesis, which is another process that is fundamental to all life. For the empirical part, we assemble a large compilation of protein:rRNA ratios in both prokaryotic and eukaryotic microbes to test the quantitative predictions of the model.

Before proceeding with the construction and analysis of the model, we point out that: (1) our goal here is not to explain the maintenance of the pattern over evolutionary time but rather to determine whether N:P = 16 has any significance for microbial cells, (2) our focus on optimal growth does not mean that we assume or suggest the prevalence of optimal conditions in the ocean, (3) our emphasis on protein:rRNA is not to say that the overall cellular N:P equals the N:P of all protein and rRNA in the cell, and (4) consideration of how different cellular pools can perturb cellular N:P from the Redfield ratio and how the feedbacks between the environment and plankton maintain the pattern in the ocean are separate questions that are beyond our scope here. Rather, our approach focuses on the biochemical and elemental stoichiometry of the ribosome/protein synthesis machinery under optimal conditions, and under N- and P-limitations.

METHODS

Empirical compilation of microbial protein:rRNA ratios

We identified relevant studies by searching ISI Web of Science and Google Scholar using combinations of key words such as 'macromolecular composition', RNA, rRNA, protein, cellular, cells, yeast, *Escherichia coli*, *Saccharomyces cerevisiae* and growth. In addition, we followed cited references in all the identified articles to find additional relevant studies. Only studies that directly measured macromolecular composition and reported either absolute protein and RNA (or rRNA) content or protein:RNA ratio were selected; studies that indirectly estimated either protein or RNA content using prior literature were excluded. The majority of studies reported growth under various limitations, in which case only data corresponding to the highest reported growth rate were selected. Studies that measured macromolecular content only under severe limitation and far from optimal growth conditions were excluded. If not reported, rRNA content was calculated as 85% of the reported total RNA content.

'Biological constants' for Table 1

The length (measured in amino acid residues) of an RNA polymerase (l_a): for a prokaryote the total number of amino acid residues in all subunits of one RNA polymerase core is *c.* 3400 (Bremer & Dennis 1996). For a eukaryote, we use the number of amino acid residues in the entire Pol I holoenzyme, which is *c.* 4800 (for consistency we also use it in the derivation of ϕ_r for a eukaryote).

The length (measured in ribonucleotides) of all rRNAs in a ribosome (l_r): the rRNA components of a prokaryotic ribosome (70S)

Table 1 Biological constants contained in eqn 6. References for all the constants and their derivations are provided in the Methods

Parameter	Description	Prokaryote	Eukaryote	Units
l_a	The length in amino acids of RNA polymerase (Pol I holoenzyme for eukaryotes)	3400	4800	aa pol^{-1}
l_r	The length in ribonucleotides of rRNA in one ribosome (in parenthesis for *Saccharomyces cerevisiae*)	4560	6860 (5470)	nt rib^{-1}
m_a	Average mass of an amino acid in peptide chains	110	110	Da aa^{-1}
m_r	Average mass of a ribonucleotide	340	340	Da nt^{-1}
n_a	Average N content in amino acids	0.17	0.17	mg mg^{-1}
n_r	Average N content in four ribonucleotides	0.15	0.15	mg mg^{-1}
p_r	Average P content in four ribonucleotides	0.09	0.09	mg mg^{-1}

are 23S, 16S and 5S containing *c.* 2900, 1540 and 120 ribonucleotides respectively. Hence, $l_r \approx 4560$ for a prokaryote (Dennis *et al.* 2004). The rRNA components of a eukaryotic ribosome (80S) are 28S, 18S, 5.8S and 5S containing *c.* 4600, 1850, 160 and 120 ribonucleotides respectively. Hence, $l_r \approx 6860$ for a eukaryote. However, the eukaryote *S. cerevisiae*, instead of 28S rRNA subunit, has 25S with *c.* 3400 nucleotides, making its $l_r \approx 5470$ (Warner 1999). While the number of ribonucleotides in any specific subunit can vary slightly, such variation has no effect on the precision of the calculation of cellular N:P in eqn 6 within two significant digits (see Appendix S2 and *Mathematica Player* file for sensitivity analyses).

Average mass of an amino acid in a polypeptide chain (m_a): the average mass of an amino acid is *c.* 119 Da (Karpinets *et al.* 2006), but that of an amino acid residue is *c.* 110 Da. As it is a polypeptide chain that is of interest here, $m_a \approx 110$ Da.

Average mass of a base in RNA chain (m_r): average over adenine, cytosine, guanine and uracil yields $m_r \approx 340$ Da.

The N content in proteins (n_a): the number of N atoms per amino acid residue varies from 1 to 4 and the average content of N in proteins is 17% by mass (Sterner & Elser 2002). Although the relative abundance of amino acids, and hence the average mass of an amino acid and N content in proteins, can vary among species (Bragg & Hyder 2004; Elser *et al.* 2006, 2010), such variation, even when taken at its extreme between different taxa, results in ± 7% variation in the number of N atoms per side chain, which yields only ± 1% variation in N mass content in proteins. Such a small variation has no effect on our calculations (see Appendix S2 for sensitivity analyses).

The N and P content in RNA (n_r and p_r, respectively): the number of N atoms per ribonucleotide varies from 2 to 5. Each ribonucleotide contains exactly one P atom. The average composition of RNA is $C_{9.5}H_{13.75}O_8N_{3.75}P$ (Geider & La Roche 2002), which yields $n_r \approx 0.15$ and $p_r \approx 0.09$. While the relative abundance of ribonucleotides in rRNA can vary among species (Bragg & Hyder 2004), such variation, even when taken at its extreme, results in $< \pm 0.5\%$ variation for N content and $< \pm 0.1\%$ for P content. Such small variations in n_a, n_r and p_r are inconsequential for our estimates of cellular N:P (see Appendix S2 for sensitivity analyses).

The derivation of parameters in Table S1 is more involved and given in Appendix S1, while sensitivity analyses can be found in Appendix S2 and a *Mathematica Player* file.

MODEL

Derivation of the homoeostatic protein:rRNA equilibrium

We assume optimal conditions for a cell culture, which means that it grows exponentially. Denoting the mass of all proteins as a and all rRNAs as r, we express the overall protein synthesis as $da/dt = \gamma r$, where γ is the rate of protein synthesis per unit of rRNA mass. The overall rRNA synthesis can be expressed as $da/dt = \psi a$, where ψ is the rate of rRNA synthesis per unit of protein mass. The intricacies of protein and rRNA synthesis are packed into parameters γ and ψ, which we later decompose into simpler biological variables and constants. For now, we note that protein-to-rRNA ratio (β) in the culture can be written as $\beta = a/r$.

As there are no extrinsically imposed constraints, then the total mass of proteins and rRNAs, $a + r$, grows exponentially, while the protein:rRNA ratio, β, is governed by the equation:

$$\frac{d\beta}{dt} = \frac{d}{dt}\left(\frac{a}{r}\right) = \left(\frac{da}{dt}r - \frac{dr}{dt}a\right)r^{-2} = \left(\gamma r^2 - \psi a^2\right)r^{-2} = \gamma - \psi\beta^2.$$

Solving $d\beta/dt = 0$ yields a unique asymptotically stable equilibrium for β; this means that independent of initial conditions, β *tends to a unique homoeostatic value*:

$$\beta = \sqrt{\gamma/\psi}. \quad (1)$$

When proteins and rRNA are in this ratio, then the newly synthesized substances are in the same ratio as in the existing biomass, which is equivalent to 'balanced growth'. This ratio also corresponds to a maximal growth for the following reason. Were β to be fixed at any value then the overall growth would be limited either by protein synthesis or rRNA synthesis. If it is the former, then the specific growth rate of the biomass equals to (protein synthesis rate)/(protein content) = $(\gamma r)/a = \gamma/\beta$. If it is the latter, then the specific growth rate equals to (rRNA synthesis rate)/(rRNA content) = $(\psi a)/r = \psi\beta$. In either case, the specific growth rate of the total mass of proteins and rRNAs can be expressed as $min\{\gamma/\beta, \psi\beta\}$. For any given translation and transcription rates (i.e. fixed γ and ψ), the maximal growth rate would be achieved at the co-limitation by the two synthesis rates, i.e. when $\gamma/\beta = \psi\beta$. This equality has the same solution for β as eqn 1, which means that the obtained unique homoeostatic protein:rRNA ratio assures *both a balanced and maximal growth*.

Derivation of the Redfield N:P equation

To quantify eqn 1, we decompose parameters γ and ψ into basic biomolecular components. For γ, we note that an active ribosome (i.e. the one to which a growing polypeptide chain is attached) synthesizes proteins at a rate equal to the average rate of peptide chain elongation (σ_a) times the average mass of an amino acid residue ($m_a \approx 110$ Da), i.e. $\sigma_a m_a$. The mass of rRNA in a ribosome is equal to the average mass of a ribonucleotide ($m_r \approx 340$ Da) times the length of rRNA measured in nucleotides (l_r), i.e. $m_r l_r$. Thus, the rate of protein synthesis per rRNA unit is $\sigma_a m_a/(m_r l_r)$. Multiplying this by the fraction of ribosomes that are actively translating (ϕ_a), yields:

$$\gamma = \phi_a \sigma_a m_a/(m_r l_r). \quad (2)$$

To determine ψ, note that an active RNA polymerase (or Pol I and Pol III in eukaryotes) synthesizes rRNAs at the rate equal to an average nascent precursor-rRNA elongation rate (σ_r) times m_r, i.e. $\sigma_r m_r$. The mass of RNA polymerase is the product of its length measured in amino acids (l_a) and m_a, i.e. $m_a l_a$. Hence, the rate of rRNA synthesis per unit of active RNA polymerase is $\sigma_r m_r/(m_a l_a)$. Multiplying it by the fraction of the total protein that is RNA polymerase actively transcribing rRNA (ϕ_r) yields:

$$\psi = \phi_r \sigma_r m_r/(m_a l_a). \quad (3)$$

There are two caveats to be considered about eqns 2 and 3. First, the effective synthesis rates can be slower because of the degradation of proteins and rRNAs. However, here we focus on nutrient replete and optimal conditions that assure maximal growth rates. Under such conditions, the degradation of proteins and rRNAs is generally negligible ($< 2.5\%\ h^{-1}$) (Russell & Cook 1995; Pratt *et al.* 2002) and thus has little effect on our calculations (see Appendix S2 in Supporting Information for our sensitivity analysis). However, we

note here that at low growth rates macromolecular turnover can become an important factor. Second, as external and internal transcribed spacers are excised from nascent precursor-rRNAs, the values for σ_r need to be lowered to account for those nucleotides that are transcribed but not incorporated into ribosomes (see Appendix S1).

From eqns 1 to 3, it follows that the homoeostatically stable protein:rRNA ratio is determined by the following formula:

$$\beta = \frac{m_a}{m_r}\sqrt{\frac{l_a\phi_a\sigma_a}{l_r\phi_r\sigma_r}}. \quad (4)$$

The N:P (θ) of the total protein and rRNA in a cell can be determined using the following three stoichiometric constants: N content averaged over the 20 amino acids (n_a), and N and P contents averaged over the four ribonucleotides (n_r and p_r, respectively), yielding the following equation:

$$\theta = (n_a a + n_r r) : (p_r r) = (n_a(a/r) + n_r) : p_r = (n_a\beta + n_r) : p_r. \quad (5)$$

From eqns 4 to 5, it follows that the homoeostatic protein:rRNA ratio corresponds to the following N:P ratio (by mass; to convert to atomic ratios one needs to multiply by *c.* 2.2):

$$\theta = \left(n_a\sqrt{\gamma/\psi} + n_r\right) : p_r = \left(n_a\frac{m_a}{m_r}\sqrt{\frac{l_a\phi_a\sigma_a}{l_r\phi_r\sigma_r}} + n_r\right) : p_r. \quad (6)$$

Parametrization of the Redfield N:P equation

Equation 6 contains eleven parameters of which (1) seven are biological constants (m_a, m_r, l_a, l_r, n_a, n_r, p_r; Table 1 and see the Methods) that not only are independent of nutrient and any other environmental conditions but also are likely applicable to nearly all organisms, and (2) four are biochemical parameters (σ_a, σ_r, ϕ_a, ϕ_r; Table 2 and see Appendix S1), the values of which can depend on growth conditions. Under optimal conditions, however, physico-chemical properties constrain three of these parameters (σ_a, σ_r, and ϕ_a) to specific values, leaving only ϕ_r without a firm theoretical bound (see Appendix S1). All four parameters, however, have been measured *in vivo* for *E. coli* and *S. cerevisiae* during optimal growth (Boehlke & Friesen 1975; Bonven & Gullov 1979; Bremer & Dennis 1996; French *et al.* 2003; Dennis *et al.* 2009; Kos & Tollervey 2010). Values and justification of all constants and parameters are provided in Supporting Information.

Substituting the values provided in Tables 1 and 2 into eqn 4 yields a homoeostatic protein:rRNA value of 3.0 for the prokaryote *E. coli* and 2.7–3.5 for the eukaryote *S. cerevisiae* (thus, a rule of thumb: the microbial protein:rRNA ratio that maximizes growth under optimal conditions is roughly 3). Remarkably, when protein and rRNA are in such ratios, then the N:P of the total protein and rRNA is at or near the Redfield value. As per eqn 5, the N:P is 16 for *E. coli* and 15–18 for *S. cerevisiae* (all calculated values throughout the article are rounded off to two significant digits unless specified otherwise).

Table 2 Parameters contained in eqn 6 and their values at optimal conditions for a prokaryote *Escherichia coli* grown at 37 °C and a eukaryote *Saccharomyces cerevisiae* grown at 30 °C. References for all parameters and their derivations are provided in Appendix S1

Parameter	Description	*E. coli*	*S. cerevisiae*	Units
σ_a	Peptide elongation rate	21	10	aa s^{-1} rib^{-1}
σ_r	Nascent precursor-rRNA elongation rate (corrected for excised ETS and ITS)	85 (71)	40–60 (33–49)	nt s^{-1} pol^{-1}
ϕ_a	Fraction of ribosomes that are actively translating	0.80	0.80–0.90	mg mg^{-1}
ϕ_r	Fraction of the total protein that is RNA polymerase actively transcribing rRNAs	0.0020	0.0018	mg mg^{-1}

Empirical analysis of microbial protein:rRNA ratio under optimal conditions

The N:P values corresponding to our theoretically obtained optimal protein:rRNA ratios are at or near the canonical Redfield N:P. To see how these values compare with empirical values, we compiled literature data on the macromolecular composition of 27 microbial species, including *E. coli* and *S. cerevisiae*, grown at optimal or near optimal conditions in 31 distinct studies (see Methods; Table S1). As shown in Table 3, despite variations in the methods for estimating macromolecular composition, the diversity of species, and the differences in growth media and conditions, the mean values for the empirically derived protein:rRNA ratios for *E. coli*, *S. cerevisiae*, and the entire data set all fall within 3 ± 0.7 range and are in good agreement with our theoretical results: 2.3 for *E. coli* (cf. our theoretically derived value of 3.0), 3.7 for *S. cerevisiae* (cf. our theoretically derived range 2.7–3.5). Averaged over all the data for the 27 species, the optimal protein:rRNA is 3.2.

These protein:rRNA ratios map via eqn 5 to their corresponding N:P ratios, which all fall around the Redfield value: N:P = 13 for *E. coli* (cf. our derived value of 16), N:P = 19 for *S. cerevisiae* (cf. our derived range 15–18) and over all the species the N:P of cellular protein + rRNA pools is 17.

The effects of N- and P-limitation on the homoeostatic N:P ratio

Our finding that the homoeostatic protein:rRNA ratio at optimal conditions has N:P *c.* 16 should not be interpreted as meaning that '16' is universally advantageous. In fact, our model predicts in what

Table 3 Summary of data from Table S1 on protein:rRNA ratios for *Escherichia coli*, *Saccharomyces cerevisiae* and 25 other species directly measured in 31 distinct studies

Species	Protein:rRNA	N:P	*n*
E. coli	2.3 ± 0.2	13 ± 1	13
S. cerevisiae	3.7 ± 0.6	19 ± 2	9
All data for the 27 species	3.2 ± 0.2	17 ± 1	51

The means of protein:rRNA ratios are rounded here to two significant digits ± the standard errors of the mean; *n* is the number of observations. We used eqn 5 to calculate the corresponding N:P ratios, which all appear to fall near the canonical Redfield N:P value of 16.

direction the ratio should shift according to the relative availability of N and P in the environment.

Specifically, the effects of N-limitation can be captured via a slowing of the synthesis of N-rich proteins, i.e. lower γ values, which according to eqn 2 can be achieved with a reduction of the peptide chain elongation rate (σ_a) and/or the fraction of ribosomes that are actively translating (ϕ_a) (note that the other three parameters in eqn 2 are 'biological constants', which are beyond any control by the organism). According to eqn 4, lower γ values result in lower homoeostatic protein:rRNA ratios. This in turn, according to eqn 6, yields N:P ratios that are below the Redfield value under N-limitation. This result is in disagreement with previous theoretical derivations that predict high structural N:P values (N:P = 37.4) for N-limited plankton (Klausmeier *et al.*, 2004).

The effects of P-limitation can be captured via a slowing of the synthesis of P-rich rRNAs, i.e. lower ψ values, which according to eqn 3 can be achieved by lowering the rRNA chain elongation rate (σ_r) and/or the fraction of protein that is active RNA polymerase (ϕ_r) (as with eqn 2, the other three parameters in eqn 3 are 'biological constants' and cannot be altered by the organism). Lower ψ values yield higher homoeostatic protein:rRNA ratios (see eqn 4). This in turn, according to eqn 6, yields N:P ratios that are above the Redfield value, an outcome applicable to N-fixers that are more likely to be P- rather than N-limited and often tend to have N:P ratios higher than the Redfield ratio (Letelier & Karl 1996).

DISCUSSION

We constructed a simple dynamic model that accounts not only for protein and rRNA synthesis but also for the ways in which these two processes are pivotal for each other. As RNAs translate proteins, proteins in the form of RNA polymerase (RNAP) in prokaryotes and the RNA polymerases Pol I and III in eukaryotes, transcribe rRNAs. We are not aware of any prior work that has applied this fact to the Redfield problem. We show that when these two processes are considered simultaneously with no imposed constraints on the amounts of protein and rRNA then, regardless of initial conditions, the protein:rRNA ratio converges to a homoeostatic value corresponding to the square root of the translation rate divided by the transcription rate (eqn 1). We further decompose this ratio into basic macromolecular constants and parameters (eqn 4). This homoeostatic ratio is 'optimal' in two ways: first, it yields a balanced growth during which the newly synthesized constituents are in the same ratio as in the biomass that generates them; second, for given translation and transcription rates, it yields the maximal possible growth rate. Thus, the first, and perhaps most important, outcome of our analysis shows that the fundamental system of protein synthesis and ribosome biogenesis itself constitutes a biochemical attractor.

We then calculate the N:P ratio of this attractor using three fundamental biomolecular 'constants': the N content averaged over the twenty amino acids and the N and P contents averaged over the four ribonucleotides (eqn 6). Furthermore and also significantly, it appears that, given biologically meaningful values of other key parameters at optimal conditions, this N:P ratio is remarkably similar to the canonical value of 16. Moreover, our theoretically derived values for this attractor protein/rRNA ratio closely match the compilation of data from 31 distinct studies. Thus, based on the above theoretical and empirical results, our answer to Falkowski's question is affirmative and we conclude that Redfield's canonical N:P ratio of 16 does have special biological significance: it is the N:P ratio of the major biochemical constituents of a microbial cell growing in balance at its maximal capacity. While various specific allocation patterns to apparatus beyond the protein synthesis system can potentially shift N:P values for different groups of organisms [e.g. vascular plants (Agren 2004)], our analysis suggests that a core protein:rRNA attractor imposes a central tendency for cellular N:P to approach 16. Indeed, the distribution of N:P ratio in photoautotrophs across aquatic and terrestrial systems shows surprising similarities (Elser *et al.* 2000; Agren 2004) highlighting the fact that all organisms balance the two fundamental processes – protein and rRNA biogenesis.

It's important to note that, although our results indicate an inherent significance for N:P *c.* 16, they do not mean that the canonical ratio is universally optimal. Under P-limited conditions, a core biochemistry with high N:P ratios is more advantageous, while N-limited conditions are more favourable to species with core biochemistry that has low N:P ratios. Interestingly, our analysis agrees in principle with previous findings (Klausmeier *et al.* 2004; Karpinets *et al.* 2006) that show that the optimality of cellular N:P ratio depends on the environment. However, our results suggest an opposite 'polarity' for N:P ratios under N- and P-limitation. While previous theoretical findings propose an advantage of organismal N:P ratios significantly higher than the Redfield ratio under *both* P- and N-limited conditions (Klausmeier *et al.* 2004), our analysis indicates that N-limited conditions should favour species with low N:P ratios, but P-limited conditions should favour species with high N:P ratios, which is consistent with a comprehensive meta-analysis of field and laboratory data that shows that organismal N:P correlates positively with N:P supply ratios (Hall *et al.* 2005). Moreover, our results are consistent with experimental findings for changes in cellular N:P under P- vs. N-limited growth conditions, despite the fact that we do not invoke storage compartments in our model (Goldman *et al.* 1979; Elrifi & Turpin 1985).

Our model and eqn 6 for the Redfield N:P ratio contain eleven parameters. Of these, seven are 'biological constants' that have very little variation across all organisms and three are parameters for which narrow bounds at optimal conditions are prescribed by physicochemical and thermodynamic constraints. This leaves only one parameter, the fraction of protein that is RNA polymerase actively transcribing rRNAs (ϕ_r), with no theoretical bounds. Constraining ϕ_r theoretically is difficult and would need to involve an entirely separate endeavour that would involve the distribution of various protein pools, including ribosomal proteins and, possibly, energy acquisition and expenditure considerations. Thus, although the value for ϕ_r taken from direct *in vivo* measurements puts our derived homoeostatic N:P value at or near the canonical N:P value, the completely theoretical derivation of N:P = 16 remains open. Nevertheless, this should not obscure our central result, which holds regardless of the actual numeric value for ϕ_r, namely, that the interdependence of two universal life processes – rRNA and protein biogenesis – leads to a homoeostatic protein:rRNA ratio. Furthermore, under optimal conditions and based on the best available data for key model parameters, this ratio also quantitatively corresponds to the canonical N:P value of 16. Our results reinforce Redfield's original argument and support the validity of subsequent biogeochemical models that rely on an inherent significance of N:P = 16 for plankton. One of the most robust and extensive stoichiometric patterns on Earth appears to be deeply rooted in the core stoichiometry of the foundational structures of life.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Parameters in Table 2 and their parameterization under optimal conditions.
Appendix S2 Sensitivity analysis for protein:rRNA and N:P ratios.
Table S1 Compilation of literature data on protein:rRNA ratio.
A Mathematica Player file for sensitivity analyses.
To open the file, please download the Mathematical Player freely available for all computer platforms here: http://www.wolfram.com/player

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