8. Root, C.M., Ko, K.I., Fafari, A., and Wang, J.W. (2011). Presynaptic facilitation by neuropeptide signaling mediates odor-driven food search. Cell *145*, 133–144.
9. Marella, S., Mann, K., and Scott, K. (2012). Dopaminergic modulation of sucrose acceptance behavior in *Drosophila*. Neuron *73*, 941–950.
10. Longden, K.D., Muzzu, T., Cook, D.J., Schultz, S.R., and Krapp, H.G. (2014). Nutritional state modulates the neural processing of motion vision. Curr. Biol. *24*, 890–895.
11. Hausen, K., and Wehrhahn, C. (1990). Neural circuits mediating visual flight control in flies. II. Separation of two control systems by microsurgical brain lesions. J. Neurosci. *10*, 351–360.
12. Chiappe, E.M., Seelig, J.D., Reiser, M.B., and Jayaraman, V. (2010). Walking modulates speed sensitivity in *Drosophila* motion vision. Curr. Biol. *20*, 1470–1475.
13. Maimon, G., Straw, A.D., and Dickinson, M.H. (2010). Active flight increases the gain of visual motion processing in *Drosophila*. Nat. Neurosci. *13*, 393–399.
14. Niell, C.M., and Stryker, M.P. (2010). Modulation of visual responses by behavioral state in mouse visual cortex. Neuron *65*, 472–479.
15. Saleem, A.B., Ayaz, A., Jeffery, K., Harris, K.D., and Carandini, M. (2013). Integration of visual motion and locomotion in mouse visual cortex. Nat. Neurosci. *16*, 1864–1869.
16. Longden, K.D., and Krapp, H.G. (2009). State-dependent performance of optic-flow processing interneurons. J. Neurophysiol. *102*, 3606–3618.
17. Bacon, J.P., Thompson, K.S., and Stern, M. (1995). Identified octopaminergic neurons provide an arousal mechanism in the locust brain. J. Neurophysiol. *74*, 2739–2743.
18. Suver, M.P., Mamiya, A., and Dickinson, M.H. (2012). Octopamine neurons mediate flight-induced modulation of visual processing in *Drosophila*. Curr. Biol. *22*, 2294–2302.
19. Burrows, M., and Pflüger, H.-J. (1995). Action of locust neuromodulatory neurons is coupled to specific motor patterns. J. Neurophysiol. *74*, 347–357.
20. Mentel, T., Duch, C., Stypa, H., Wegener, G., Müller, U., and Pflüger, H.J. (2003). Central modulatory neurons control fuel selection in flight muscle of migratory locust. J. Neurosci. *23*, 1109–1113.

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Microbial Ecology: Finding Structure in the Rare Biosphere

Protists (unicellular eukaryotes) play important roles in marine ecosystems but are tremendously diverse and many remain uncharacterized. Deep-sequencing of a universal marker gene has helped resolve community composition patterns among rare and abundant protistan sequence groups in coastal European waters.

Charles Bachy[1] and Alexandra Z. Worden[1,2,*]

Understanding organismal diversity and its role in ecosystem stability is a long-standing pursuit of the ecological sciences. No matter the domain of life — archaea, bacteria, or eukaryotes — or nature of the taxa under study — unicellular or multicellular — the grail is to connect diversity and relative abundance (i.e., community composition) with functional ecology. Of course, methods used between the macro- and microbial ecology research domains are different. In macroecology, diversity can generally be assessed using morphological characteristics and connection to ecosystem roles is readily apparent. In microbial ecology, diversity assessments are almost entirely dependent on molecular signatures, e.g., nucleic acid sequences derived from the ribosomal RNA (rRNA) gene present in all cellular life. This is because morphological features can be inconsistent across evolutionary relationships, or lacking, and thus are not necessarily valuable indicators of microbial diversity [1]. Hence, we move from tangible differences in community structure to a world defined by phylogenetic analyses or clustered sequence similarity groups (operational taxonomic units, OTUs) [2]. A new study by Logares *et al.* [3] reported in this issue of *Current Biology* describes patterns that emerge when marine microbial eukaryotic communities are parsed along the lines of rare and abundant OTUs and compared across European coastal sites.

Increasing attention has been placed on understanding the properties of rare versus abundant components of microbial communities. Even a rocket scientist will see value in studying the abundant members of a community — in protistan communities these are often photosynthetic organisms — with some taxa having unique features that allow their blooms to be discriminated from space [4]. What is meant by the terms ‘abundant’ or ‘rare’ is of course subjective. Typically ‘rare species’ are defined as belonging to the long tail in rank-abundance curves used to depict diversity and often termed ‘the rare biosphere’ [5,6]. The blossoming of rare biosphere research is directly linked to the advent of high-throughput gene marker sequencing, referred to as tag or bar-coded sequencing [6,7]. Until these methods were available it was difficult to study the rare biosphere. While we could get hints of which taxa were rare, we could not explore them with statistical rigor. Hence, cross-site comparisons held little meaning because the results could simply reflect under-sampling, where the chance of getting any particular rare sequence is random, not significant. Deep-sequencing has changed this ball game and investigators can now assess diversity, as well as the rare biosphere, with greater power.

Why is exploration of the rare biosphere important? We still do not know the extent to which taxa in the rare biosphere play significant roles in community function. This is important because one proposed characteristic of these communities is that they contain considerable functional redundancy, i.e., overlapping ecosystem functions or niches. Such ‘redundancy’ is perceived by some as a security net for inevitable extinctions or the demise of taxa — although this topic requires a much bigger debate than can be taken on in 1,200 words. It is hypothesized that rare eukaryotic microbes can become dominant under changing environmental conditions and that the functional redundancy (theoretically) represented by these taxa allows biogeochemical processes to be maintained even

under dramatic perturbations [8]. With climate-change-induced perturbations looming it would be good to know whether the rare biosphere does indeed provide such a stabilizing force. Of course some rare taxa may not become dominant, but could still have key, as yet unrecognized, ecological roles in microbial communities — akin to keystone species. Understanding these organisms and their phenotypes will be critical for determining the abiotic and biotic factors that shape community composition.

The considerable nature of the rare biosphere was first highlighted for prokaryotes [5,6]. Bacterial diversity surveys then demonstrated rare taxa could become dominant under favorable conditions, leading to the term 'seed bank' as a descriptor for these microbes [9]. Of course alongside these reports came studies on inflation of the rare biosphere caused by higher sequencing error rates (inherent to some deep-sequencing platforms) and short sequences. These studies provide methods to minimize artefactual diversity [10,11] and discuss advantages of phylogenetic approaches over OTU analyses [7,12]. With time, sequence numbers become larger and larger, and in spite of more stringent quality control filtering steps, there is still potential overestimation of rare species. Nevertheless, the presence of an immense number of rare protistan sequences in each sample investigated is now well documented [13–15]. However, community structure and the relationship between rare and abundant OTUs is not well understood.

Logares *et al.* [3] move the field forward by comparing marine communities in samples from six European coastal locations. They start with close to six million sequences derived from RNA (converted to cDNA) in addition to sequences sampled directly from DNA. The sequences come from 23 surface water samples that represent three cell-size fractions sampled at each of the six sites (two sites were sampled twice). The sites span a broad range of temperatures (10 to 23°C) and salinities (16 to 38). Together the samples are analyzed as regional (all 6 sites combined) versus local (geographic sites as far north and south as Oslo and Naples, respectively, as well as the Black Sea) and comparisons are made between OTUs categorized as locally abundant (>1%) or rare (<0.01%), or regionally abundant (>0.1%) or rare (<0.001). The size and breadth of the study offers a unique point of view on ecological patterns. Abundant sub-communities appear to be structured by cell size while the rare sub-communities are grouped by geographical location. These observations can be further tested with improved replication, use of a spectrum of PCR primers, and attention to how rRNA operon copy number varies between taxa (an under-investigated but hugely important source of variation and artefacts when grouping OTUs). Furthermore, Logares *et al.* [3] found that up to 30% of sequences per sample belonged to neither the rare nor abundant categories. Who and what functions does this significant intermediate fraction of the community represent? Are these microbes stable intermediates or in 'transition' between rate and abundant forms? Greater spatial–temporal resolution (estimates provided were that 64 to 67% of OTUs in the European coastal region were sampled) will help answer these questions and ensure patterns seen for rare sub-communities do not result from under-sampling issues.

Importantly, Logares *et al.* [3] provide evidence for a dramatic divergence between prokaryotic and eukaryotic microbes in terms of ribosomal activity levels within the rare biosphere. For prokaryotes, some OTUs seen using DNA are less frequently detected using transcripts, suggesting dormancy [9]. Less is known for eukaryotes, but a study of two lakes showed dormancy was more important in shaping bacterial than eukaryotic microbial communities [16]. Using more than an order of magnitude higher OTU sampling, and a much more comprehensive sample set, Logares *et al.* [3] demonstrate a 1:1 correspondence in relative abundances based on DNA:RNA. This implicates the eukaryotic rare biosphere as a ribosomally active suite of organisms, potentially important to ecosystem function in real-time, not just when taxa manage to proliferate.

As Logares *et al.* highlight in their introduction "…limited knowledge of diversity and community structure across space and time hinders our understanding of the links between microbial life and ecosystem functioning" [3]. We now know considerably more about spatial patterns in diversity of microbial eukaryotes in surface coastal waters. But can rare today mean abundant tomorrow? Transitions between the rare and abundant realms are influenced by the fourth dimension, time, and related seasonal changes. These patterns remain ill-characterized, especially for the rare biosphere. In freshwater environments, protistan communities undergo continual reassembly of abundant species (and overall community composition) with time, or associated parameters, as a driver [17,18]. A great part of the natural variability in microbial communities, protistan and prokaryotic alike, is associated with temporal dynamics [19] in addition to factors as simple as depth [20]. Thus, defining the marine 'rare biosphere' will require temporal resolution akin to that performed in 'time-series' studies with cruises at approximately monthly intervals. Beyond issues of sampling design, what is the link between the incredible diversity of protists and ecological function? Interestingly, Logares *et al.* [3] do not mention the name of a single organism in their study; this is because bridging the gap between an OTU and taxonomic or phenotypic data is extremely difficult. An OTU cannot necessarily be considered representative of a single functional role, or even to have a defined trophic mode. Thus, unlike the state of affairs for macroecology, the significance of patterns in rare and abundant communities will not speak to ecosystem function, or resilience, until functional roles are captured for the great diversity of protistan taxa and a robust mechanism is resolved for assigning OTUs to biological and ecological phenotypes.

References

1. Atlas, R.M. (1984). Diversity of microbial communities. In Advances in Microbial Ecology, Volume 7, K.C. Marshall, ed. (Boston: Springer US), pp. 1–47.
2. Caron, D.A., Countway, P.D., Savai, P., Gast, R.J., Schnetzer, A., Moorthi, S.D., Dennett, M.R., Moran, D.M., and Jones, A.C. (2009). Defining DNA-based operational taxonomic units for microbial-eukaryote ecology. Appl. Environ. Microbiol. *75*, 5797–5808.
3. Logares, R., Audic, S., Bass, D., Bittner, L., Boutte, C., Christen, R., Claverie, J.-M., Decelle, J., Dolan, J.R., Dunthorn, M., *et al.* (2014). Patterns of rare and abundant marine microbial eukaryotes. Curr. Biol. *24*, 813–821.

4. Read, B.A., Kegel, J., Klute, M.J., Kuo, A., Lefebvre, S.C., Maumus, F., Mayer, C., Miller, J., Monier, A., Salamov, A., *et al.* (2013). Pan genome of the phytoplankton *Emiliania* underpins its global distribution. Nature *499*, 209–213.
5. Sogin, M.L., Morrison, H.G., Huber, J.A., Mark Welch, D., Huse, S.M., Neal, P.R., Arrieta, J.M., and Herndl, G.J. (2006). Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc. Natl. Acad. Sci. USA *103*, 12115–12120.
6. Pedros-Alio, C. (2012). The rare bacterial biosphere. Annu. Rev. Mar. Sci. *4*, 449–466.
7. Vergin, K.L., Beszteri, B., Monier, A., Cameron Thrash, J., Temperton, B., Treusch, A.H., Kilpert, F., Worden, A.Z., and Giovannoni, S.J. (2013). High-resolution SAR11 ecotype dynamics at the Bermuda Atlantic Time-series Study site by phylogenetic placement of pyrosequences. ISME J. *6*, 481–492.
8. Caron, D.A., and Countway, P.D. (2009). Hypotheses on the role of the protistan rare biosphere in a changing world. Aquat. Microb. Ecol. *57*, 227–238.
9. Lennon, J.T., and Jones, S.E. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. Nat. Rev. Microbiol. *9*, 119–130.
10. Huse, S.M., Welch, D.M., Morrison, H.G., and Sogin, M.L. (2010). Ironing out the wrinkles in the rare biosphere through improved OTU clustering. Environ. Microbiol. *12*, 1889–1898.
11. Quince, C., Lanzen, A., Davenport, R.J., and Turnbaugh, P.J. (2011). Removing noise from pyrosequenced amplicons. BMC Bioinformatics *12*, 38.
12. Koeppel, A.F., and Wu, M. (2013). Surprisingly extensive mixed phylogenetic and ecological signals among bacterial Operational Taxonomic Units. Nucleic Acids Res. *41*, 5175–5188.
13. Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D., Breiner, H.W., and Richards, T.A. (2010). Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol. Ecol. *19* (*Suppl 1*), 21–31.
14. Stoeck, T., Behnke, A., Christen, R., Amaral-Zettler, L., Rodriguez-Mora, M.J., Chistoserdov, A., Orsi, W., and Edgcomb, V.P. (2009). Massively parallel tag sequencing reveals the complexity of anaerobic marine protistan communities. BMC Biol. *7*, 72.
15. Lecroq, B., Lejzerowicz, F., Bachar, D., Christen, R., Esling, P., Baerlocher, L., Osteras, M., Farinelli, L., and Pawlowski, J. (2011). Ultra-deep sequencing of foraminiferal microbarcodes unveils hidden richness of early monothalamous lineages in deep-sea sediments. Proc. Natl. Acad. Sci. USA *108*, 13177–13182.
16. Jones, S.E., and Lennon, J.T. (2010). Dormancy contributes to the maintenance of microbial diversity. Proc. Natl. Acad. Sci. USA *107*, 5881–5886.
17. Mangot, J.F., Domaizon, I., Taib, N., Marouni, N., Duffaud, E., Bronner, G., and Debroas, D. (2013). Short-term dynamics of diversity patterns: evidence of continual reassembly within lacustrine small eukaryotes. Environ. Microbiol. *15*, 1745–1758.
18. Nolte, V., Pandey, R.V., Jost, S., Medinger, R., Ottenwalder, B., Boenigk, J., and Schlotterer, C. (2010). Contrasting seasonal niche separation between rare and abundant taxa conceals the extent of protist diversity. Mol. Ecol. *19*, 2908–2915.
19. Chow, C.E., Sachdeva, R., Cram, J.A., Steele, J.A., Needham, D.M., Patel, A., Parada, A.E., and Fuhrman, J.A. (2013). Temporal variability and coherence of euphotic zone bacterial communities over a decade in the Southern California Bight. ISME J. *7*, 2259–2273.
20. Not, F., Gausling, R., Azam, F., Heidelberg, J.F., and Worden, A.Z. (2007). Vertical distribution of picoeukaryotic diversity in the Sargasso Sea. Environ. Microbiol. *9*, 1233–1252.

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Protein Translocation: The Sec61/SecYEG Translocon Caught in the Act

The Sec61/SecYEG complex mediates both the translocation of newly synthesized proteins across the membrane and the integration of transmembrane segments into the lipid bilayer. New cryo-electron microscopy studies show ribosome–channel complexes in action and reveal their repertoire of conformational states.

Martin Spiess

Biological membranes separate cellular compartments, generating and preserving concentration gradients and electrical potentials. How are entire polypeptides transported across or inserted into membranes while maintaining the barrier? This task is accomplished by a conserved protein-conducting channel — the SecYEG complex at the plasma membrane of prokaryotes, or the Sec61 translocon at the endoplasmic reticulum of eukaryotes [1,2]. Ribosomes translating secretory or membrane proteins are targeted to the translocon by signal peptides. Hydrophilic sequences are threaded through a polar channel, while apolar transmembrane (TM) segments stop further translocation and are laterally released into the lipid bilayer. From extensive biochemical analyses and crystal structures of the closed, idle translocon, a general picture of these dynamic processes has been pieced together. Two new studies [3,4] now show cryo-electron microscopy (EM) structures of translocons in action, arrested either at the point of signal sequence insertion, polypeptide translocation, or transmembrane segment integration, letting us watch the translocon at work more directly than ever. This work confirms that the picture that emerged from previous biochemical data is encouragingly accurate.

As a hydrophobic signal sequence emerges from the translating ribosome, it is bound by the signal recognition particle (SRP) and targeted to SRP receptors in the membrane. The ribosome binds to cytosolic loops of the translocon, whereupon the signal sequence mediates pore opening and initiates transfer of the growing polypeptide from the ribosome through the channel. Hydrophobic segments trigger lateral opening of the channel and integrate into the membrane as TM segments. Exactly how these steps work mechanistically is not known.

The translocon is composed of subunits SecY, E, and G in bacteria with ten, one, and one or two TM domains, respectively, corresponding to Sec61α, γ, and β in eukaryotes [1]. The first crystal structure of an idle translocon, from *Methanocaldococcus jannaschii* 10 years ago [5], changed the view of the translocation pore dramatically. Rather than an oligomer of several Sec complexes forming a wide water-filled channel, it was found to be a compact helix bundle of a single heterotrimer with the potential to open a narrow pore (Figure 1A). The ten TM segments of SecY form an hourglass shape with an empty vestibule on the cytosolic side and a lumenal cavity occupied by a short hydrophobic helix — the so-called plug. The two cavities are separated by a central constriction of six apolar amino acid side chains. SecY appears to be composed of two