

# Energetic Evolution of Cellular Transportomes

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## Abstract

Transporter proteins mediate the translocation of substances across the membranes of living cells. We performed a genome-wide analysis of the compositional reshaping of cellular transporters (the transportome) across the kingdoms of bacteria, archaea, and eukarya. We show that the transportomes of eukaryotes evolved strongly towards a higher energetic efficiency, as ATP-dependent transporters diminished and secondary transporters and ion channels proliferated. This change has likely been important in the development of tissues performing energetically costly cellular functions. The transportome analysis also indicated seven bacterial species, including *Neorickettsia risticii* and *Neorickettsia sennetsu*, as likely origins of the mitochondrion in eukaryotes, due to the restricted presence therein of clear homologues of modern mitochondrial solute carriers.

**Keywords:** energetic efficiency, evolution, cellular membrane, mitochondria, transporters

## Introduction

The expansion of life on Earth has involved competition among organisms for limited energy resources [1]. Arguments have been made in favour of growth rate over growth efficiency in organisms competing within a specific niche [2], which implies a natural selection towards an improved ability to capture and utilize the available energy sources for survival and reproduction [3,4]. Darwinian evolutionary theory originally covered only phenotypic improvements at the organismal level, but we nowadays also recognize the importance of molecular and cellular events such as the acquisition of mitochondria by eukaryotes. This enabled an increase of eukaryotic cell size and complexity due to a more “efficient” generation of the cellular fuel ATP [5,6]. Cells

need to allocate considerable resources to energize their transportomes. For example, brain neurons account for approximately 20% of the basal metabolic rate in humans, mostly for the movement of ions across neuronal membranes [7]. In general, a metabolic cost of up to 60% of the total ATP requirement in organisms is estimated for the activity of their transportomes [8,9]. Thus, it would be reasonable to hypothesize that an improved energetic performance of the transportome might be selected for over the course of evolution.

Despite the importance of cellular transportomes (also reported as the second largest component of the human membrane proteome [10]), transporters are surprisingly

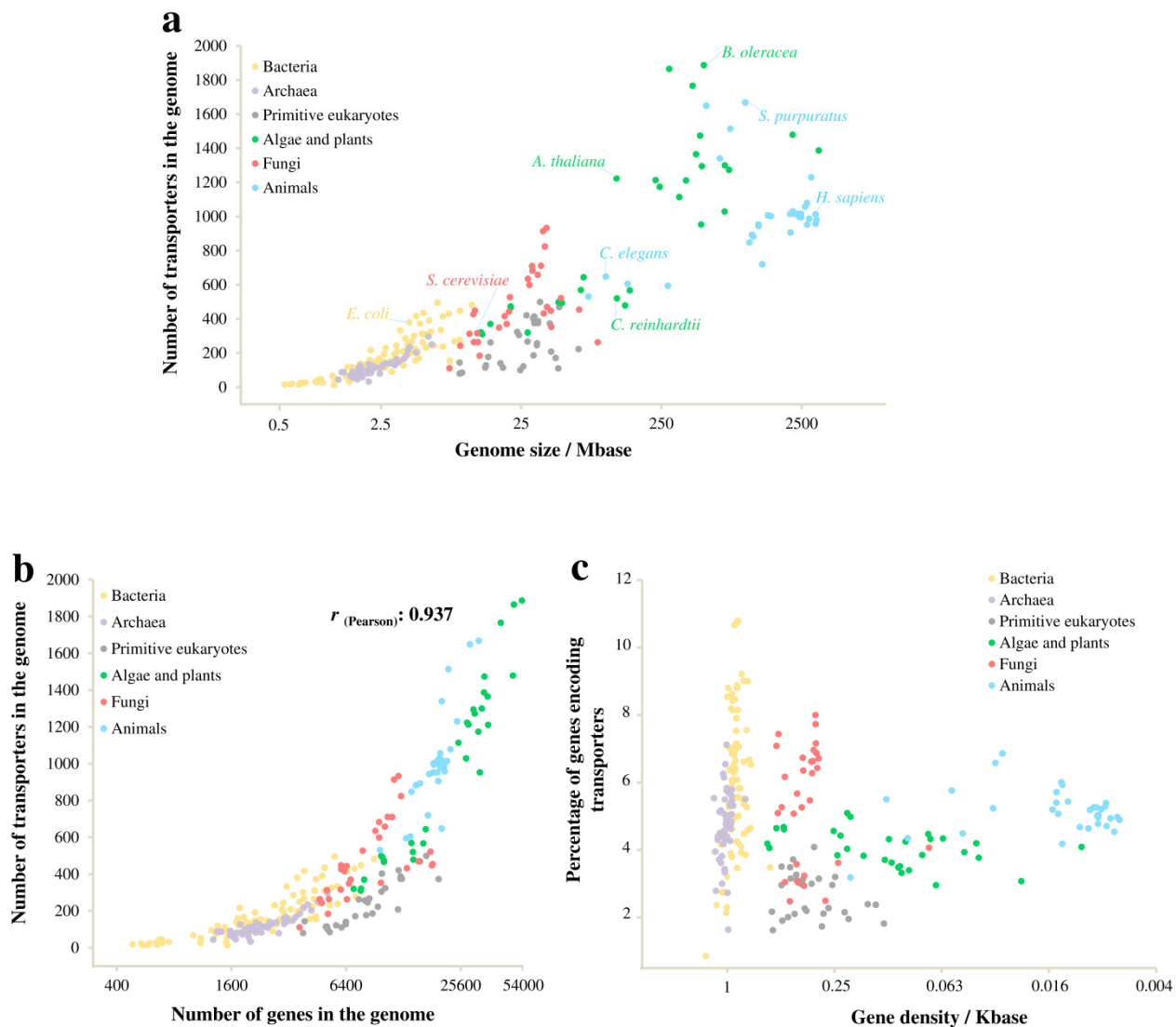
understudied [11]. Additionally, the presence of substrate-binding proteins as the partners of a subset of membrane transporters [12] makes the cellular transport machinery more complicated than if we consider only the transporters themselves. The tightening of porous and leaky primordial envelopes such that they did not let in (and could learn to efflux) all kinds of substances [13,14] has been proposed as a turning point for membrane transporters to co-evolve together with lipid bilayer membranes [15]. Different classes of transporters, each including several transporter superfamilies, share a common ancestral family of peptides which carry 1-4 transmembrane domains and form homo- and hetero-oligomer channels [16–19]. Intragenic duplication and triplication have been the major events promoting the diversification of transporter proteins [17,19]. Classical evolutionary theory on the basis of natural selection proposed by Charles Darwin [3,4] explains how the random variability of the genome as the diversification force has given the chance for specialisation, improved performance, and adaptation to the continuously changing biosphere with limited energy resources. Here, we therefore annotated the cellular transportomes of bacteria, archaea and eukarya and analysed their composition with a focus on the energetic efficiency. The analyses include all the three classes of transporters, *i.e.*, ion channels, secondary transporters, and ATP-dependent transporters. To translocate substrates, ATP-dependent transporters bind and hydrolyse ATP [20], ion channels form pores for selective diffusion of ions and small molecules, and the secondary transporters shuttle substrate molecules across biological membranes either through energy independent facilitated diffusion or via exploiting membrane electrochemical potential gradients through uniport, symport and antiport [21].

## Results and Discussion

To study the compositional changes of transportomes, we analysed the transportomes of 249 evolutionarily distant species (of which 222 were annotated in this study) from archaea, bacteria and eukarya. These included 126 prokaryotic species (from 16 taxonomic phyla and 60 taxonomic orders), 30 primitive eukaryotes (different species from Alveolata, Kinetoplastida and Amoebozoa), 30 algal and plant species, 30 fungal species, and 33 animal species (See S1 Data). The transportomes were annotated using the Transporter Automatic Annotation Pipeline at TransportDB [22]. Notably, the species had large differences in the size of both their genomes and their transportomes (Fig 1a, b and S1 Data).

We found that the sizes of an organism's transportome tends to increase with its genome size, though there are considerable intra-kingdom variations (Fig 1a) (see also [23]). In particular, primitive eukaryotes have small transportomes with 100-500 members, relative to their genome sizes of tens of Mb when compared to the bacteria with modestly sized genomes (less than 10 Mb). Ion channels, secondary transporters, and primary active transporters were found in each of the analysed domains of life (See S1 Data). This indicates a very early appearance for these three classes of transporters, possibly in a common ancestor.

In agreement with previous reports [24,25], the genome size had a higher rate of enlargement than did the gene number, resulting in a decreased gene density over the course of evolution (Fig 1c). The transportome enlargement was found to be well correlated with the increase in the gene number ( $r_{\text{Pearson}} = 0.937$ ) with the exception of primitive eukarya, where the transportome-



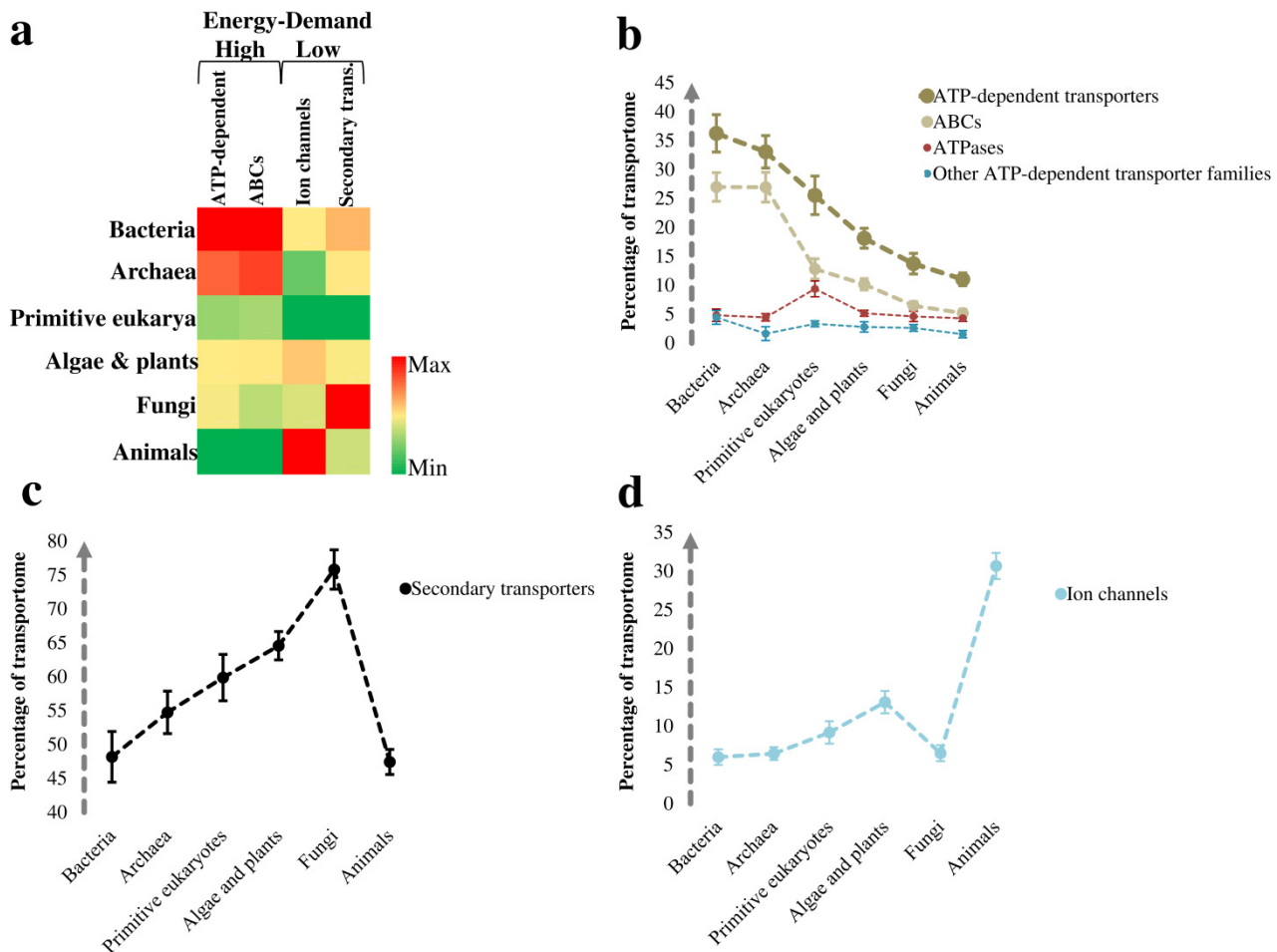
**Fig 1. Transportomes differ in size among species and evolutionarily distant domains of life. (a)** The number of membrane transporters per organism in relation to the genome size. **(b)** The number of membrane transporters per genome in relation to the number of total genes. **(c)** Percentage of transporter-coding genes in relation to gene density.

encoding proportion of genes was the lowest (Fig 1b, c). Of most interest, the composition of the transportomes changed from prokaryotes to eukaryotes and also among the eukaryotic kingdoms along with the transportome enlargement (Fig 2a). Specifically, we found higher intra-genomic frequencies (frequency relative to the total

number of genes in the genome) of ATP-dependent transport classes in prokaryotes than eukaryotes. An opposing trend was found for low-energy-demanding transport classes. This indicates different rates of gene proliferation among the evolved transporter classes; low-energy-demanding transporter families have expanded at

a higher rate. Notably, the transportomes of primitive eukaryotes also represent a transition state between

prokaryotic and higher eukaryotic kingdoms (Fig 2a).



**Fig 2. The evolutionary dynamics of transportomes composition.** (a) Heat-map representation of the changes in the number of members of the transporter classes. To calculate the intra-genomic frequencies, the numbers of transporter members are normalized to the total number of genes per genome. The heat-map is drawn for each class of ion channels, secondary transporters, and ATP-dependent transporters and therefore colors are not comparable between the classes. (b) The fraction of ATP-dependent transporters in the transportomes. All of the variations of ATP-dependent transporters and ABC superfamily except the difference between bacteria and archaea are significant at 0.1% level. (c) The fraction of secondary transporters in the transportomes. Only the difference between bacteria and animals is not statistically significant ( $p = 0.635$ ). (d) The fraction of ion channels in the transportomes. All differences, except among fungi, bacteria and archaea, are significant with a  $p$ -value less than 0.001. The values on panels b-d are shown as mean  $\pm$  t-test based 99% confidence intervals. The variations were also confirmed on the arc sin  $\sqrt{x}$  transformed data (See S1 Data). The family names of the transporters can also be found in the S1 Data.

We further compared the prevalence of secondary transporters, ion channels, and ATP-dependent transporters within the transportomes of each species, and averaged these over the larger taxonomic groups (Fig 2b-d). In general, we observed compositional changes that indicate a positive adaptive selection of the no- to low-cost-flux equilibrative ion channels and carriers, and negative selection of the energetically more expensive ABC transporters. More specifically, we found significant variations in each of the transporter classes (Fig 2b-d). While ca. 27% of all the bacterial and archaeal transporters are ABC transporters, this fraction decreases to 13% in primitive eukaryotes, 10% in algae and plants and a mere 5-6% in fungi and animals (Fig 2b). On the other hand, an increased contribution to the cellular transportome was found for secondary transporters in eukaryotes, particularly in fungi (Fig 2c). Furthermore, animals with  $\approx 30\%$  and the green domain of life including algae and plants with  $\approx 13\%$  showed higher percentages of ion channels when compared to the bacteria and archaea with only 6%-7% (Fig 2d). During evolution, and in parallel with the genome enlargement and gene pool expansion, each of the three classes of

transporters had a chance to contribute proportionally to the expansion of transportomes. By contrast, our results show a preference for the low-energy demanding transporters (ion channels and carriers) over the energy-costly transporter classes (ATP-dependent families, and ABCs in particular) in the transition from prokaryotes to eukaryotes. We defined the energy usage efficiency of a transportome (EUE) as the average required energy per single substrate translocation. We calculated the EUE values for the transportomes studied (more details in the Methods section). The EUE describes the overall energetic performance of transportomes at organismal level and most importantly it does not indicate the total energy consumption by the cellular transportome, because the latter depends also on the flux through individual transporters that is largely unknown. In contrast to the total energy requirements, the EUE is therefore not subjected to spatiotemporal variations.

By comparing the average EUEs of the transportomes across the different domains of life, we found that the EUE has improved in eukaryotes by reductions of up to 0.50 ATP in the average ATP consumption per single transport event mediated by transporters (Table 1).

**Table 1. Improvement in the energy-usage efficiency ( $\Delta$ EUE) calculated as changes in the average ATP-usage per single transport cycle.**

Domains of life	Bacteria	Archaea	Primitive eukaryotes	Algae and plants	Fungi	Animals
Bacteria	0					
Archaea	-0.03	0				
Primitive eukaryotes	-0.16	-0.14	0			
Algae and plants	-0.27	-0.25	-0.11	0		
Fungi	-0.30	-0.27	-0.13	-0.02	0	
Animals	-0.49	-0.46	-0.32	-0.21	-0.19	0

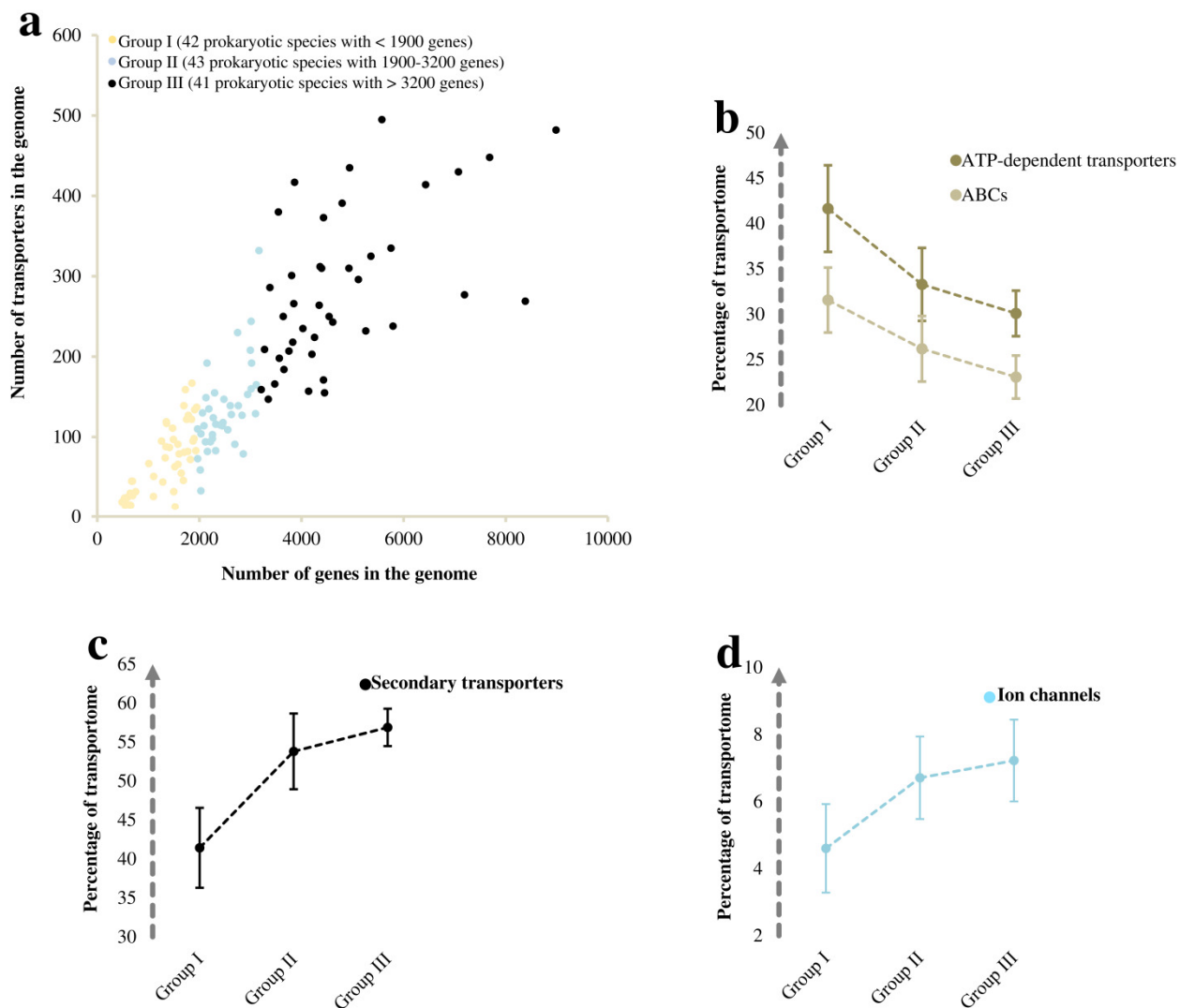
The changes ( $\Delta$ EUE) are calculated as  $\text{ATP-usage}_{\text{domain of life in the matrix-row}} - \text{ATP-usage}_{\text{domain of life in the matrix-column}}$  (see methods). Negative changes represent the reduction in ATP-usage and improved EUE of transportomes.

Furthermore, the data suggest that animals have mainly relied on the diversification of ion channels, fungi on secondary transporters, and finally, algae and plants on both transporter classes for the evolution of their transportomes (Fig 2b-d). For energetically efficient transportomes, organisms therefore adopted different strategies, likely due to their specialisations and different requirements. The trend of expansion of ion channels and secondary transporters at the expense of ATP-dependent transporters also holds true for the prokaryotic transportomes (Fig 3), even though they did not undergo the kind of intensive developmental specialisation as was required in multicellular eukaryotes.

The analyses also indicate family member expansions for secondary transporters (from 60-100 in prokaryotes to more than 400 in animals and 600 in plants in average) and ion channels (from 10 members in prokaryotes to more than 300 members in animals). To find prokaryotic and eukaryotic specific families, we further enriched our analysis by including the publicly-available data for the secondary transporters and ion channels of prokaryotes found at the TransportDB 2.0 (<http://www.membranetransport.org/transportDB2/index.html>). This extended our prokaryotes to 2637 transportomes (S1 Table). We also included two eukaryotic transportomes from diatoms found at TransportDB 2.0 (S1 Table). When comparing the organisms for the presence of different transporter families, we found that eight secondary transporter families were completely lost in the passage to eukaryotes (See S1 Data). Additionally, we found that five new families of secondary transporters had emerged in eukaryotes (See S1 Data). Specifically, we did not find any prokaryotic hits in GenBank for these eukaryotic families, that must have diverged massively

[26] from some ancestral genes. In contrary to secondary transporters, ion channels have evolved through both intra-family expansions and the substantial appearance of new families. While there were only seven families specific to the prokaryotes, 18 eukarya-specific families were found for ion channels (See S1 Data). Importantly, the mitochondria-specific solute carriers, *i.e.*, solute carrier family SLC25 [27,28], are absent from the transportomes of all 143 archaeal species (See S1 Table for the list of organisms). Among the bacteria, including 259 alpha-proteobacteria, of which 69 belonged to the order *Rickettsiales*, we found only seven bacterial genomes that encoded members of the mitochondrial transporter family. These are *Neorickettsia risticii*, *Neorickettsia sennetsu*, *Legionella pneumophila*, *Legionella longbeachae*, *Acidaminococcus intestini*, *Cardinium endosymbiont* and *Butyrivibrio proteoclasticus*. The presence of the mitochondrial transporter family members indicates that these seven bacterial species are possible origins of the mitochondrion in eukaryotes. The first two species are Gram-negative obligatory intracellular bacteria from the order *Rickettsiales*, an order that in previous studies was proposed (based on different evidence) as the most likely origin of the mitochondrion in eukaryotes [26–29].

A surprising insight here was related to the five evolutionarily younger secondary transporter families found only in eukaryotes (See S1 Data): three of these, about which we have experimental information, were energy-independent and low-energy-demanding transporter families for choline, silicate, and vitamin A. The last two belong to the 4 TMS multidrug endosomal transporter and chloroplast maltose exporter families. The choline transporter-like family is involved in choline



**Fig 3. The compositional changes in the transportomes of prokaryotes with different genome sizes. (a)** Comparison between the transportome size and total gene number among prokaryotes including bacteria and archaea. All of the 126 studied species are clustered into three groups based on the total number of the genes. **(b)** The fraction of ATP-dependent transporters in the transportomes. **(c)** The fraction of secondary transporters in the transportomes. **(d)** The fraction of ion channels in the transportomes. The values on panels b-d are shown as mean  $\pm$  t-test based 99% confidence interval. The variations were also confirmed on the arc sin  $\sqrt{x}$  transformed data (See S1 Data). Group I and III differ significantly for all of the transporter classes with a  $p$ -value of  $< 0.001$ .

influx [33]. The 'birth' of a cheap and sodium-independent transporter for choline is important because of the broad cellular usage of choline. Choline is an essential precursor for membranes and for the

neuromodulator acetylcholine [34,35]. The silicon transporter family is also energetically cheap and has a silicate:sodium symport stoichiometry of 1:1 [36]. The animal-specific vitamin A receptor/transporter (STRA6)



mediates costless influx and efflux of vitamin A derivatives by a mechanism not seen in any other transporter class [37,38]. Of particular interest, the animal visual system is dependent on vitamin A and has a substantial energetic cost, *e.g.*, up to 15% of resting metabolism in the Mexican fish *Astyanax mexicanus* [39,40]. Such photo-detection involves the single-photon-triggered isomerization of 11-*cis*-retinal to all-*trans*-retinal, which must be recycled back through efflux and influx steps of these isomers between the retinal pigmented epithelial and photoreceptor cells [37,41–43]. Thus, the evolved energy-independent membrane translocation of the vitamin A isomers seems to be an adaptive trait for higher energetic performance. This is in line with the positive selection reported for STRA6 in different mammalian phyla [44]. Additionally, we found a higher representation of ion channels in the animal kingdom when compared to the other domains of life (Fig 2d). The membrane transport of ions is important for highly energy-demanding sensory tissues [7,45] and it can therefore be hypothesized that the extensive diversification of ion channels and the costless transport of vitamin A in animals are trade-offs between the benefits of the evolved nervous and visual systems and their high energy requirements. In conclusion, our results provide genome-scale molecular evidence to the evolution of the cellular transportome towards an improved energetic efficiency.

## Materials and Methods

The publicly-available membrane transporter data on ion channels and secondary transporters were extracted from TransportDB (<http://www.membranetransport.org/transportDB2/>) [22]. The transportomes of 126 prokaryotic species (78 bacteria and 48 archaea) and 96 eukaryotic species (22 primitive eukaryotes, 24 algae and plants, 23

fungi, and 27 animals) (See S1 Data) were annotated using the Transporter Automatic Annotation Pipeline at TransportDB [22]. We also included 27 eukaryotic species including transportomes of 8 primitive eukaryotes, 6 algae and plants, 7 fungi, and 6 animals publicly available at TransportDB (See S1 Data). To study the compositional changes of transportomes, we did not include any transportomes from prokaryotes publicly available at the TransportDB. This is due to the incomplete information on the ABC transporters. The majority of ABC transporters in prokaryotes are coded by different genes of an operon, where each gene codes for different subunits [46], and these should be excluded from the data and considered as single transporter in our analyses on the transportome composition. However, this information on the ABC coding genes and operons is not provided for the publicly-available transportomes of prokaryotes.

To predict the transportomes, the proteomes of organisms were downloaded from the Genbank and Ensembl databases (<http://www.ensembl.org/index.html>, <http://fungi.ensembl.org/index.html>, <http://protists.ensembl.org/index.html>, <http://plants.ensembl.org/index.html>) [47]. All proteins with fewer than 100 amino acids were excluded. Taken together, we analysed 78 bacterial, 48 archaeal, 30 primitive eukaryotes, 30 algal and plant, 30 fungal, and 33 animal transportomes, each representing one independent biological replicate (per species). The list of organisms with their genome size and total number of transporters is shown in S1 Data and S1 Table. The annotated transportomes were manually filtered for multi-prediction hits as well as the alternative isoforms of transport proteins before the analysis. Taken together, our analyses on the transporter families included all of the 15 ATP-dependent transporter families, 51 ion channel



families, and 90 secondary transporter families which were present in the studied organisms. We used Student's t-test to examine the possible differences among the samples, *i.e.*, domains of life. The data were in the format of counts and percentages. Therefore, to exclude a possible divergence from normality, we also performed the analyses on the arc sin  $\sqrt{x}$  transformed data. All the analyses can be found in S1 Data.

To make an approximation of the energetic performance of the cellular transportomes, we calculated the inter-transportome changes in energy-usage efficiency (EUE), which we defined as the average free energy demand for a single transport event. The following assumptions were made when calculating EUE values. Equilibrative ion channels require no free energy for the transport action. While some of the secondary transporters also act in an energy-independent manner known as equilibrative transport or facilitated diffusion, others exploit the electrochemical potential established by ATPases across membranes [13,21,48–50]. For active transport, secondary transporters are considered to use membrane electrochemical potential gradients, coupled to a varying stoichiometry (0.5 to 3) of ions per turnover [49,51–72]. Considering the stoichiometry of  $\approx$  2-3 ions pumped per ATP hydrolysed by ATPases [73–82], even concentrative secondary transporters would not use more than 0.5 ATP per substrate translocation across a membrane on average. The average of two substrates per ATP tends, therefore, to be conservative for secondary transporters and a higher rate of substrate translocation can also be expected. In contrast, the ATP-dependent members belonging to the ABC superfamily, and also the mitochondrial protein translocase, the type III secretory

pathway, the chloroplast envelope protein translocase, and the arsenite-antimonite efflux families, generally show a 1:2 stoichiometry of substrate:ATP hydrolysis [83–89]. To calculate the inter-transportome variations in the energy-usage efficiency ( $\Delta$ EUE), we therefore applied the equation:

$$\Delta\text{EUE} (\Delta\text{ATP-usage per single transport cycle}) = [2_{\text{ATP}} \times \Delta\% \text{ADT} + 0.5_{\text{ATP}} \times \Delta\% \text{ST} + 0.0_{\text{ATP}} \times \Delta\% \text{IC}] / 100$$

where ADT, ST, and IC are ATP-dependent transporters, secondary transporters, and ion-channels, respectively. The F/V/A-type ATPases and ATP synthases were excluded from the ATP-dependent transporters. This is because they provide the energy as ATP or membrane electrochemical potential for the rest of the transporters. It is worthy of note that the efficiency of energy usage indicates the average energy demand per unit of action. So the expression and activity levels of transporters are not taken into the account when calculating the EUE. A more general example would compare two organisms with exactly the same transportome but with differences in the expression levels of transportome members among them. Here, the EUE of transportome machineries of the two organisms are equal since they use exactly same transportome machinery. This is thus irrespective of the differences in activity levels of transporters, which define the total energy demand for the given transportome.

## Abbreviations

ADT, ATP-dependent transporters; EUE, energy-usage efficiency; IC, ion-channels; ST, secondary transporters.

## Availability of data

The datasets supporting the conclusions of this article are included within the article and Supplementary Data 1.

## Supplementary Information

**Supplementary Table 1:** The list of organisms with publicly-available data on the ion channels and secondary transporters.

**Supplementary Data 1:** Genomic and transcriptome data on the organisms included in the study.

## Author contributions

BD, DBK, and IB conceived the study. BD performed the analyses by consulting DBK and IB. BD, DBK, and IB wrote the manuscript.

## Competing financial interests

The authors declare no competing financial interest.

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## References

1. Demetrius L. Thermodynamics and Evolution. *J Theor Biol.* 2000;206: 1–16. doi:10.1006/jtbi.2000.2106
2. Westerhoff HV, Hellingwerf KJ, Van Dam K. Thermodynamic efficiency of microbial growth is low but optimal for maximal growth rate. *Proc Natl Acad Sci U S A.* 1983;80: 305–309.
3. Darwin C. On the Origin of Species by Means of Natural Selection, Or, The Preservation of Favoured Races in the Struggle for Life. J. Murray; 1859.
4. Stanley SM. A theory of evolution above the species level. *Proc Natl Acad Sci.* 1975;72: 646–650.
5. Martin W, Müller M. The hydrogen hypothesis for the first eukaryote. *Nature.* 1998;392: 37–41. doi:10.1038/32096
6. Lane N, Martin W. The energetics of genome complexity. *Nature.* 2010;467: 929–934. doi:10.1038/nature09486
7. Niven JE, Laughlin SB. Energy limitation as a selective pressure on the evolution of sensory systems. *J Exp Biol.* 2008;211: 1792–1804. doi:10.1242/jeb.017574
8. Verduyn C, Stouthamer AH, Scheffers WA, van Dijken JP. A theoretical evaluation of growth yields of yeasts. *Antonie Van Leeuwenhoek.* 1991;59: 49–63.
9. Skou JC. The Identification of the Sodium–Potassium Pump (Nobel Lecture). *Angew Chem Int Ed.* 1998;37: 2320–2328. doi:10.1002/(SICI)1521-3773(19980918)37:17<2320::AID-ANIE2320>3.0.CO;2-2
10. Almén MS, Nordström KJ, Fredriksson R, Schiöth HB. Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin. *BMC Biol.* 2009;7: 50. doi:10.1186/1741-7007-7-50
11. César-Razquin A, Snijder B, Frappier-Brinton T, Isserlin R, Gyimesi G, Bai X, et al. A Call for Systematic Research on Solute Carriers. *Cell.* 2015;162: 478–487. doi:10.1016/j.cell.2015.07.022
12. Bosdriesz E, Magnúsdóttir S, Bruggeman FJ, Teusink B, Molenaar D. Binding proteins enhance specific uptake rate by increasing the substrate–transporter encounter rate. *FEBS J.* 2015;282: 2394–2407. doi:10.1111/febs.13289
13. Kell DB, Oliver SG. How drugs get into cells: tested and testable predictions to help discriminate between transporter-mediated uptake and lipoidal bilayer diffusion. *Front Pharmacol.* 2014;5: doi:10.3389/fphar.2014.00231
14. Kell DB, Swainston N, Pir P, Oliver SG. Membrane transporter engineering in industrial biotechnology and whole cell biocatalysis. *Trends Biotechnol.* 2015;33: 237–246. doi:10.1016/j.tibtech.2015.02.001
15. Mulikjanian AY, Galperin MY, Koonin EV. Co-evolution of primordial membranes and membrane

- proteins. *Trends Biochem Sci.* 2009;34: 206–215. doi:10.1016/j.tibs.2009.01.005
16. Perland E, Fredriksson R. Classification Systems of Secondary Active Transporters. *Trends Pharmacol Sci.* 2017;38: 305–315. doi:10.1016/j.tips.2016.11.008
17. Saier MH. Tracing pathways of transport protein evolution. *Mol Microbiol.* 2003;48: 1145–1156.
18. Saier MH. Transport protein evolution deduced from analysis of sequence, topology and structure. *Curr Opin Struct Biol.* 2016;38: 9–17. doi:10.1016/j.sbi.2016.05.001
19. Shimizu T, Mitsuke H, Noto K, Arai M. Internal gene duplication in the evolution of prokaryotic transmembrane proteins. *J Mol Biol.* 2004;339: 1–15. doi:10.1016/j.jmb.2004.03.048
20. Dean M, Annilo T. Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. *Annu Rev Genomics Hum Genet.* 2005;6: 123–142. doi:10.1146/annurev.genom.6.080604.162122
21. Forrest LR, Krämer R, Ziegler C. The structural basis of secondary active transport mechanisms. *Biochim Biophys Acta.* 2011;1807: 167–188. doi:10.1016/j.bbabi.2010.10.014
22. Elbourne LDH, Tetu SG, Hassan KA, Paulsen IT. TransportDB 2.0: a database for exploring membrane transporters in sequenced genomes from all domains of life. *Nucleic Acids Res.* 2017;45: D320–D324. doi:10.1093/nar/gkw1068
23. Kell DB, Dobson PD, Oliver SG. Pharmaceutical drug transport: the issues and the implications that it is essentially carrier-mediated only. *Drug Discov Today.* 2011;16: 704–714. doi:10.1016/j.drudis.2011.05.010
24. Lynch M, Conery JS. The Origins of Genome Complexity. *Science.* 2003;302: 1401–1404. doi:10.1126/science.1089370
25. Hou Y, Lin S. Distinct Gene Number-Genome Size Relationships for Eukaryotes and Non-Eukaryotes: Gene Content Estimation for Dinoflagellate Genomes. *PLoS ONE.* 2009;4. doi:10.1371/journal.pone.0006978
26. Höglund PJ, Nordström KJV, Schiöth HB, Fredriksson R. The Solute Carrier Families Have a Remarkably Long Evolutionary History with the Majority of the Human Families Present before Divergence of Bilaterian Species. *Mol Biol Evol.* 2011;28: 1531–1541. doi:10.1093/molbev/msq350
27. Palmieri F. The mitochondrial transporter family SLC25: identification, properties and physiopathology. *Mol Aspects Med.* 2013;34: 465–484. doi:10.1016/j.mam.2012.05.005
28. Hediger MA, Cléménçon B, Burrier RE, Bruford EA. The ABCs of membrane transporters in health and disease (SLC series): Introduction. *Mol Aspects Med.* 2013;34: 95–107. doi:10.1016/j.mam.2012.12.009
29. Andersson SG, Zomorodipour A, Andersson JO, Sicheritz-Pontén T, Alsmark UC, Podowski RM, et al. The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. *Nature.* 1998;396: 133–140. doi:10.1038/24094
30. Gray MW. Mitochondrial evolution. *Cold Spring Harb Perspect Biol.* 2012;4: a011403. doi:10.1101/cshperspect.a011403
31. Gray MW. Mosaic nature of the mitochondrial proteome: Implications for the origin and evolution of mitochondria. *Proc Natl Acad Sci U S A.* 2015;112: 10133–10138. doi:10.1073/pnas.1421379112
32. Wang Z, Wu M. An integrated phylogenomic approach toward pinpointing the origin of mitochondria. *Sci Rep.* 2015;5: 7949. doi:10.1038/srep07949
33. Taguchi C, Inazu M, Saiki I, Yara M, Hara N, Yamanaka T, et al. Functional analysis of [methyl-(3)H]choline uptake in glioblastoma cells: Influence of anti-cancer and central nervous system drugs. *Biochem Pharmacol.* 2014;88: 303–312. doi:10.1016/j.bcp.2014.01.033
34. Traiffort E, O'Regan S, Ruat M. The choline transporter-like family SLC44: properties and roles in human diseases. *Mol Aspects Med.* 2013;34: 646–654. doi:10.1016/j.mam.2012.10.011
35. Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a neuromodulator: cholinergic

- signaling shapes nervous system function and behavior. *Neuron*. 2012;76: 116–129. doi:10.1016/j.neuron.2012.08.036
36. Knight MJ, Senior L, Nancolas B, Ratcliffe S, Curnow P. Direct evidence of the molecular basis for biological silicon transport. *Nat Commun*. 2016;7: 11926. doi:10.1038/ncomms11926
37. Kawaguchi R, Zhong M, Kassai M, Ter-Stepanian M, Sun H. STRA6-catalyzed vitamin A influx, efflux, and exchange. *J Membr Biol*. 2012;245: 731–745. doi:10.1007/s00232-012-9463-1
38. Chen Y, Clarke OB, Kim J, Stowe S, Kim Y-K, Assur Z, et al. Structure of the STRA6 receptor for retinol uptake. *Science*. 2016;353. doi:10.1126/science.aad8266
39. Zhong M, Kawaguchi R, Kassai M, Sun H. Retina, retinol, retinal and the natural history of vitamin A as a light sensor. *Nutrients*. 2012;4: 2069–2096. doi:10.3390/nu4122069
40. Moran D, Softley R, Warrant EJ. The energetic cost of vision and the evolution of eyeless Mexican cavefish. *Sci Adv*. 2015;1: e1500363. doi:10.1126/sciadv.1500363
41. Vitamin A Metabolism in Rod and Cone Visual Cycles. *Annu Rev Nutr*. 2012;32: 125–145. doi:10.1146/annurev-nutr-071811-150748
42. Kiser PD, Golczak M, Maeda A, Palczewski K. Key enzymes of the retinoid (visual) cycle in vertebrate retina. *Biochim Biophys Acta BBA - Mol Cell Biol Lipids*. 2012;1821: 137–151. doi:10.1016/j.bbalip.2011.03.005
43. Sahu B, Maeda A. Retinol Dehydrogenases Regulate Vitamin A Metabolism for Visual Function. *Nutrients*. 2016;8. doi:10.3390/nu8110746
44. Wu J, Xiang H, Qi Y, Yang D, Wang X, Sun H, et al. Adaptive Evolution of the STRA6 Genes in Mammalian. *PLOS ONE*. 2014;9: e108388. doi:10.1371/journal.pone.0108388
45. Glynn IM. Annual review prize lecture. “All hands to the sodium pump.” *J Physiol*. 1993;462: 1–30.
46. Moussatova A, Kandt C, O’Mara ML, Tieleman DP. ATP-binding cassette transporters in *Escherichia coli*. *Biochim Biophys Acta BBA - Biomembr*. 2008;1778: 1757–1771. doi:10.1016/j.bbamem.2008.06.009
47. Kersey PJ, Allen JE, Armean I, Boddu S, Bolt BJ, Carvalho-Silva D, et al. Ensembl Genomes 2016: more genomes, more complexity. *Nucleic Acids Res*. 2016;44: D574–580. doi:10.1093/nar/gkv1209
48. Baldwin SA. Mammalian passive glucose transporters: members of an ubiquitous family of active and passive transport proteins. *Biochim Biophys Acta*. 1993;1154: 17–49.
49. Monné M, Palmieri F. Antiporters of the mitochondrial carrier family. *Curr Top Membr*. 2014;73: 289–320. doi:10.1016/B978-0-12-800223-0.00008-6
50. Mendes P, Oliver SG, Kell DB. Fitting Transporter Activities to Cellular Drug Concentrations and Fluxes: Why the Bumblebee Can Fly. *Trends Pharmacol Sci*. 2015;36: 710–723. doi:10.1016/j.tips.2015.07.006
51. Accardi A, Miller C. Secondary active transport mediated by a prokaryotic homologue of Cl<sup>-</sup> channels. *Nature*. 2004;427: 803–807. doi:10.1038/nature02314
52. Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, et al. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature*. 1997;388: 482–488. doi:10.1038/41343
53. Deng D, Yan N. GLUT, SGLT, and SWEET: Structural and mechanistic investigations of the glucose transporters. *Protein Sci Publ Protein Soc*. 2016;25: 546–558. doi:10.1002/pro.2858
54. Steel A, Nussberger S, Romero MF, Boron WF, Boyd CA, Hediger MA. Stoichiometry and pH dependence of the rabbit proton-dependent oligopeptide transporter PepT1. *J Physiol*. 1997;498 ( Pt 3): 563–569.
55. Boron VF, Hediger MA, Boulpaep EL, Romero MF. The renal electrogenic Na<sup>+</sup>:HCO<sup>3-</sup> cotransporter. *J Exp Biol*. 1997;200: 263–268.

56. Bröer S, Bröer A, Schneider HP, Stegen C, Halestrap AP, Deitmer JW. Characterization of the high-affinity monocarboxylate transporter MCT2 in *Xenopus laevis* oocytes. *Biochem J.* 1999;341 (Pt 3): 529–535. doi:10.1016/B978-0-12-394316-3.00008-9
57. Jennings ML, Adame MF. Direct estimate of 1:1 stoichiometry of K(+)-Cl(-) cotransport in rabbit erythrocytes. *Am J Physiol Cell Physiol.* 2001;281: C825–832.
58. Kahle KT, Rinehart J, Lifton RP. Phosphoregulation of the Na-K-2Cl and K-Cl cotransporters by the WNK kinases. *Biochim Biophys Acta.* 2010;1802: 1150–1158. doi:10.1016/j.bbadis.2010.07.009
59. Zhao C, Noskov SY. The molecular mechanism of ion-dependent gating in secondary transporters. *PLoS Comput Biol.* 2013;9: e1003296. doi:10.1371/journal.pcbi.1003296
60. Forster IC, Hernando N, Biber J, Murer H. Phosphate transporters of the SLC20 and SLC34 families. *Mol Aspects Med.* 2013;34: 386–395. doi:10.1016/j.mam.2012.07.007
61. Schweikhard ES, Ziegler CM. Amino acid secondary transporters: toward a common transport mechanism. *Curr Top Membr.* 2012;70: 1–28. doi:10.1016/B978-0-12-394316-3.00001-6
62. Bürzle M, Hediger MA. Functional and physiological role of vitamin C transporters. *Curr Top Membr.* 2012;70: 357–375. doi:10.1016/B978-0-12-394316-3.00011-9
63. Forster IC, Hernando N, Biber J, Murer H. Phosphate transport kinetics and structure-function relationships of SLC34 and SLC20 proteins. *Curr Top Membr.* 2012;70: 313–356. doi:10.1016/B978-0-12-394316-3.00010-7
64. Adijanto J, Philp NJ. The SLC16A family of monocarboxylate transporters (MCTs)—physiology and function in cellular metabolism, pH homeostasis, and fluid transport. *Curr Top Membr.* 2012;70: 275–311. doi:10.1016/B978-0-12-394316-3.00009-0
65. Terada T, Inui K. Recent advances in structural biology of peptide transporters. *Curr Top Membr.* 2012;70: 257–274. doi:10.1016/B978-0-12-394316-3.00008-9
66. Raja M, Puntheeranurak T, Hinterdorfer P, Kinne R. SLC5 and SLC2 transporters in epithelial cellular role and molecular mechanisms. *Curr Top Membr.* 2012;70: 29–76. doi:10.1016/B978-0-12-394316-3.00002-8
67. Markovich D. Sodium-sulfate/carboxylate cotransporters (SLC13). *Curr Top Membr.* 2012;70: 239–256. doi:10.1016/B978-0-12-394316-3.00007-7
68. Cordat E, Reithmeier RAF. Structure, function, and trafficking of SLC4 and SLC26 anion transporters. *Curr Top Membr.* 2014;73: 1–67. doi:10.1016/B978-0-12-800223-0.00001-3
69. Hendus-Altenburger R, Kragelund BB, Pedersen SF. Structural dynamics and regulation of the mammalian SLC9A family of Na<sup>+</sup>/H<sup>+</sup> exchangers. *Curr Top Membr.* 2014;73: 69–148. doi:10.1016/B978-0-12-800223-0.00002-5
70. Schnetkamp PPM, Jalloul AH, Liu G, Szerencsei RT. The SLC24 Family of K<sup>+</sup>-Dependent Na<sup>+</sup>-Ca<sup>2+</sup> Exchangers: Structure–Function Relationships. In: Bevensee MO, editor. *Current Topics in Membranes.* Academic Press; 2014. pp. 263–287. doi:10.1016/B978-0-12-800223-0.00007-4
71. Schweigel-Röntgen M, Kolisek M. SLC41 Transporters—Molecular Identification and Functional Role. In: Bevensee MO, editor. *Current Topics in Membranes.* Academic Press; 2014. pp. 383–410. doi:10.1016/B978-0-12-800223-0.00011-6
72. Döring B, Lütteke T, Geyer J, Petzinger E. The SLC10 carrier family: transport functions and molecular structure. *Curr Top Membr.* 2012;70: 105–168. doi:10.1016/B978-0-12-394316-3.00004-1
73. Sitsel O, Grønberg C, Autzen HE, Wang K, Meloni G, Nissen P, et al. Structure and Function of Cu(I)- and Zn(II)-ATPases. *Biochemistry (Mosc).* 2015;54: 5673–5683. doi:10.1021/acs.biochem.5b00512
74. Davies JM, Hunt I, Sanders D. Vacuolar H(+)-pumping ATPase variable transport coupling ratio



- controlled by pH. *Proc Natl Acad Sci U S A*. 1994;91: 8547–8551.
75. Finbow ME, Harrison MA. The vacuolar H<sup>+</sup>-ATPase: a universal proton pump of eukaryotes. *Biochem J*. 1997;324 ( Pt 3): 697–712.
76. Hernández A, Cooke DT, Clarkson DT. Effects of abnormal-sterol accumulation on *Ustilago maydis* plasma membrane H<sup>+</sup>-ATPase stoichiometry and polypeptide pattern. *J Bacteriol*. 1998;180: 412–415.
77. Cross RL, Müller V. The evolution of A-, F-, and V-type ATP synthases and ATPases: reversals in function and changes in the H<sup>+</sup>/ATP coupling ratio. *FEBS Lett*. 2004;576: 1–4. doi:10.1016/j.febslet.2004.08.065
78. Tomashek JJ, Brusilow WS. Stoichiometry of energy coupling by proton-translocating ATPases: a history of variability. *J Bioenerg Biomembr*. 2000;32: 493–500.
79. Warncke J, Slayman CL. Metabolic modulation of stoichiometry in a proton pump. *Biochim Biophys Acta*. 1980;591: 224–233.
80. Perlin DS, San Francisco MJ, Slayman CW, Rosen BP. H<sup>+</sup>/ATP stoichiometry of proton pumps from *Neurospora crassa* and *Escherichia coli*. *Arch Biochem Biophys*. 1986;248: 53–61.
81. Sosa A, Celis H. H<sup>+</sup>/PPi stoichiometry of membrane-bound pyrophosphatase of *Rhodospirillum rubrum*. *Arch Biochem Biophys*. 1995;316: 421–427. doi:10.1006/abbi.1995.1056
82. Petersen J, Förster K, Turina P, Gräber P. Comparison of the H<sup>+</sup>/ATP ratios of the H<sup>+</sup>-ATP synthases from yeast and from chloroplast. *Proc Natl Acad Sci U S A*. 2012;109: 11150–11155. doi:10.1073/pnas.1202799109
83. Patzlaff JS, van der Heide T, Poolman B. The ATP/substrate stoichiometry of the ATP-binding cassette (ABC) transporter OpuA. *J Biol Chem*. 2003;278: 29546–29551. doi:10.1074/jbc.M304796200
84. Locher KP. Mechanistic diversity in ATP-binding cassette (ABC) transporters. *Nat Struct Mol Biol*. 2016;23: 487–493. doi:10.1038/nsmb.3216
85. Rensing C, Ghosh M, Rosen BP. Families of soft-metal-ion-transporting ATPases. *J Bacteriol*. 1999; 181: 5891–5897.
86. Yang H-C, Fu H-L, Lin Y-F, Rosen BP. Pathways of arsenic uptake and efflux. *Curr Top Membr*. 2012;69: 325–358. doi:10.1016/B978-0-12-394390-3.00012-4
87. Balsera M, Soll J, Buchanan BB. Redox extends its regulatory reach to chloroplast protein import. *Trends Plant Sci*. 2010;15: 515–521. doi:10.1016/j.tplants.2010.06.002
88. Minamino T, Shimada M, Okabe M, Saijo-Hamano Y, Imada K, Kihara M, et al. Role of the C-terminal cytoplasmic domain of FlhA in bacterial flagellar type III protein export. *J Bacteriol*. 2010;192: 1929–1936. doi:10.1128/JB.01328-09
89. Herrmann JM. Converting bacteria to organelles: evolution of mitochondrial protein sorting. *Trends Microbiol*. 2003;11: 74–79.