Shifts in metabolic scaling, production, and efficiency across major evolutionary transitions of life

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The diversification of life involved enormous increases in size and complexity. The evolutionary transitions from prokaryotes to unicellular eukaryotes to metazoans were accompanied by major innovations in metabolic design. Here we show that the scalings of metabolic rate, population growth rate, and production efficiency with body size have changed across the evolutionary transitions. Metabolic rate scales with body mass superlinearly in prokaryotes, linearly in protists, and sublinearly in metazoans, so Kleiber's 3/4 power scaling law does not apply universally across organisms. The scaling of maximum population growth rate shifts from positive in prokaryotes to negative in protists and metazoans, and the efficiency of production declines across these groups. Major changes in metabolic processes during the early evolution of life overcame existing constraints, exploited new opportunities, and imposed new constraints.

energetic constraints | production efficiency | r_{max} | endosymbiosis | multicellularity

The 3.5 billion year history of life on earth was characterized by dramatic increases in the size, complexity, and diversity of living things. The first organisms were microbes with relatively simple body plans and metabolic networks. A few major transitions in form and function occurred during the subsequent evolution of life (1). The resulting diversity of contemporary organisms ranges from minute, relatively simple unicellular prokaryotes to giant, complex animals and plants containing multiple differentiated organelles, cells, tissues, and organs.

Two of the largest transitions were from simple prokaryotic to complex eukaryotic cells, and from unicellular to multicellular eukaryotes. Each transition required the integration of multiple individual organisms into a new higher-level unit of organization and selection (1, 2). These transitions involved dramatic changes in structure and function, and several orders of magnitude increase in body size (3). As all organisms share a common set of molecules and biochemical reactions (4, 5), the increases in size and organizational complexity were accomplished by assembling these universal components in new ways (6). Major changes in genetic systems made these transitions possible (1, 2), and complementary changes in metabolic systems supplied the energy and materials to grow larger and support more complex morphologies and physiologies (7, 8).

Scaling relations offer powerful insights into the fundamental processes that constrain and regulate biological structure and function. Nearly all characteristics of organisms, from use of energy to the population growth it fuels, vary with body size. Most of the variation can be described by allometric equations or power functions of the following form:

$$Y = Y_0 M^{\alpha} \quad [1]$$

where Y is the trait of interest, Y_0 is a normalization constant, M is body mass, and α is the scaling exponent. There is a large and longstanding literature on these biological scaling relations in plants and animals but that are fewer focused on unicellular prokaryotes and protists. The large changes in structure and function that occurred at the major evolutionary transitions likely affected the allometric scaling of three traits that we consider in the subsequent sections.

Metabolic Rate. Metabolic rate, B, the rate of energy transformation within an organism, is perhaps the most fundamental biological rate. It sets the pace of life. It is statistically correlated with and functionally linked to many other traits. In the 1930s, Max Kleiber (9) showed that the metabolic rate of birds and mammals scales as approximately the 3/4 power of body mass. Subsequent findings of similar scalings for metabolic rates in many kinds of life forms led to the canonization of "Kleiber's law": an α of approximately 0.75 was thought to apply to all organisms, including unicellular prokaryotes and eukaryotes (10–13). Renewed interest in biological scaling relations has led to reevaluation of Kleiber's law, with much discussion about the exact value of α in different taxonomic and functional groups. Theoretical models have attributed 3/4-power scaling to the fractal-like designs of vascular systems of large, complicated organisms (14), whereas empirical studies have reported exponents greater than 0.75 for some small unicellular organisms, animals, and plants (15–18). Clearly, the scaling of metabolic rate with body mass in small organisms needs to be reexamined, with a focus on the evolutionary transitions that connects these disparate forms of life.

Population Growth Rate. The rate of population growth, r, is another trait with fundamental importance in both ecology, in which it provides a standardized estimate of the population-level rate of biomass production, and evolution, in which it is often taken as a measure of fitness. Maximal population growth rate under optimal conditions, r_{max}, has received considerable attention in both basic and applied studies of microorganisms. Because production of new biomass for both growth and reproduction is fueled by metabolism, it has generally been assumed that r_{max} scales in the same way as mass-specific metabolic rate, so with an exponent of -0.25, given that they follow Kleiber's law. This has generally been supported by empirical studies of large, multicellular organisms (12, 19). Although a seminal early study of r_{max} in protists reached similar conclusions (20), the scaling of r_{max} across the evolutionary transitions should be reexamined.

Efficiency of Biomass Production. Another basic characteristic of organisms is the efficiency with which they convert metabolic energy into new biomass. This efficiency, E, can be expressed in units of gJ^{-1} as the rate of biomass production divided by the rate of metabolism, both standardized as per unit body mass as follows:

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$$E = r_{\max}/(B/M) \quad [2]$$

E is not only a fundamental biological parameter; it has important practical applications in areas such as agriculture, biotechnology, and biofuel production. So it is timely to quantify the scaling of E as a function of body size and across the evolutionary transitions.

Here we compile data on the scaling of these three fundamental characteristics, metabolic rate, B, maximum population growth rate, r_{max}, and efficiency of biomass production, E, in three functional groups of heterotrophic organisms: prokaryotes, protists, and small multicellular aquatic animals (hereafter "metazoans"; *SI Text*). Application of a scaling framework is especially powerful and informative when the organisms vary in body size by many orders of magnitude in body mass. Our data include organisms spanning approximately 16 orders of magnitude in body size and representing the evolutionary transitions from prokaryotes to unicellular eukaryotes to multicellular animals. To control for the effects of food supply and activity, the metabolic rate data are classified into two categories according to the conditions under which the measurements were taken: (*i*) active and fed and (*ii*) inactive or endogenous or starved. We refer to these as active and inactive. The data include 167 and 188 species in each state, respectively. We analyze these data in the context of allometric scaling to evaluate our hypothesis that scaling of metabolic rate changed across the evolutionary transitions from small, simple prokaryotes to much larger and more complex metazoans. By using nested ANOVAs, we identify differences in scaling slopes and intercepts among groups. Our findings contradict current dogma about the scaling of metabolism and $r_{\max}$, demonstrate how existing constraints and new innovations affected the evolutionary transitions, and suggest a role for energy in the diversification of life.

Results and Discussion

Whole-organism metabolic rate increases with body size across prokaryotes, protists, and metazoans, but each group is characterized by a distinctive scaling relationship that is unique to the body size range of the group (Fig. 1). Although the entire dataset for each metabolic state can be fit with a single power law that accounts for most of the variation, the relationship between body mass and metabolic rate for both active and inactive states is significantly improved by incorporating evolutionary group (ANOVA comparing a three-line with a one-line model; active, $F_{4,161} = 9.57$, $P < 0.0001$; inactive, $F_{4,182} = 6.07$, $P = 0.0001$). We also tested for differences in slopes between protists and metazoans, which differ for both active and inactive rates (ANOVA comparing a two-line with a one-line model; active, $F_{1,119} = 3.87$, $P = 0.05$; inactive, $F_{1,63} = 3.96$; $P = 0.05$). Fig. 1 shows the raw data, fits, and exponents (±SE) for each group. The slopes for the two physiological states are parallel. There is a pronounced shift in the scaling of both active and inactive metabolic rates, from highly superlinear ($\alpha = 1.7$ and 2.0) in prokaryotes, to nearly linear ($\alpha = 1.0$ and 1.1) in protists, to sublinear ($\alpha = 0.76$ and 0.79; i.e., approximately Kleiber's law) in metazoans.

The differences across groups and the large discrepancy between the canonical $\alpha = 0.75$ and the observed, significantly larger, exponents for protists and especially for prokaryotes clearly show that Kleiber's law, long thought to extend across all living things, does not hold for single-celled organisms. These data suggest that the scaling of metabolic rate is not governed by a single, overarching design principle that applies to all living things, but instead by different constraints at different body sizes and levels of structural and functional organization.

The scaling of $r_{\max}$ also changes across the evolutionary transitions. $r_{\max}$ increases with mass in prokaryotes and scales negatively in both protists and metazoans (Fig. 2*A*). This result contradicts previous findings that found $r_{\max}$ scaling with an exponent of approximately −0.25 across diverse taxa from prokaryotes to mammals (20). As metabolic rate fuels biomass production and population growth, the naive expectation is that $r_{\max}$ should scale similarly to active mass-specific metabolic rate, so as $M^{\alpha-1}$. Overall, the scalings of $r_{\max}$ roughly parallel the scalings of mass-specific active metabolic rate as expected, with no significant differences in slopes (ANOVA, $F_{3,331} = 0.13$; P value not significant; Fig. 2*A*). This supports the interpretation that metabolism fuels biomass production.

From these parallel scalings of $r_{\max}$ and mass-specific metabolic rate, it follows that the efficiency of biomass production, measured as the ratio of these two variables, is invariant with size within groups. Indeed, the efficiency of production shows no size dependence within groups. Importantly, however, the mean efficiency decreases with each successive transition, from 23×10^{-4} gJ^{-1} for

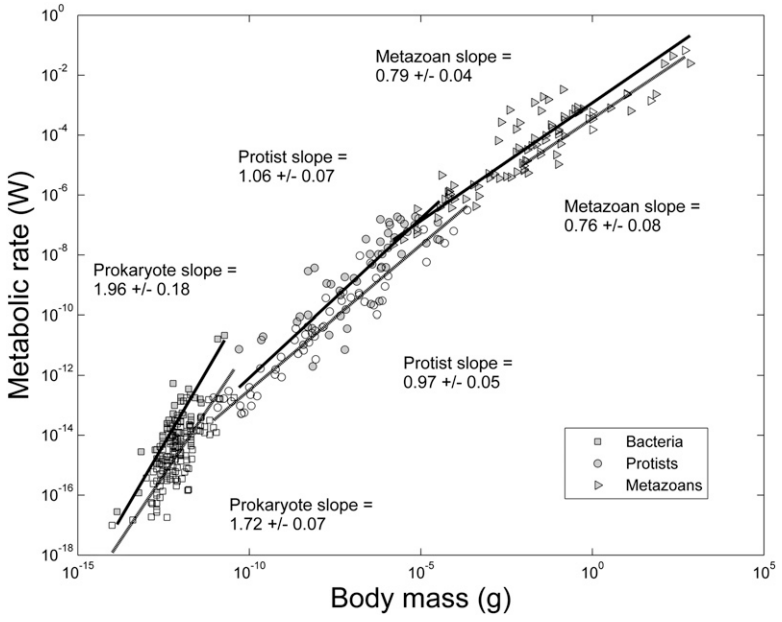

Fig. 1. Relationship between whole organism metabolic rate and body mass for heterotrophic prokaryotes, protists, and metazoans plotted on logarithmic axes. Fits are RMA slopes ± SE. Data for active (filled symbols, solid line) and inactive (unfilled symbols, gray line) metabolic rates are shown. Differences in slopes among all groups are significant for both physiological states ($P \leq 0.05$).

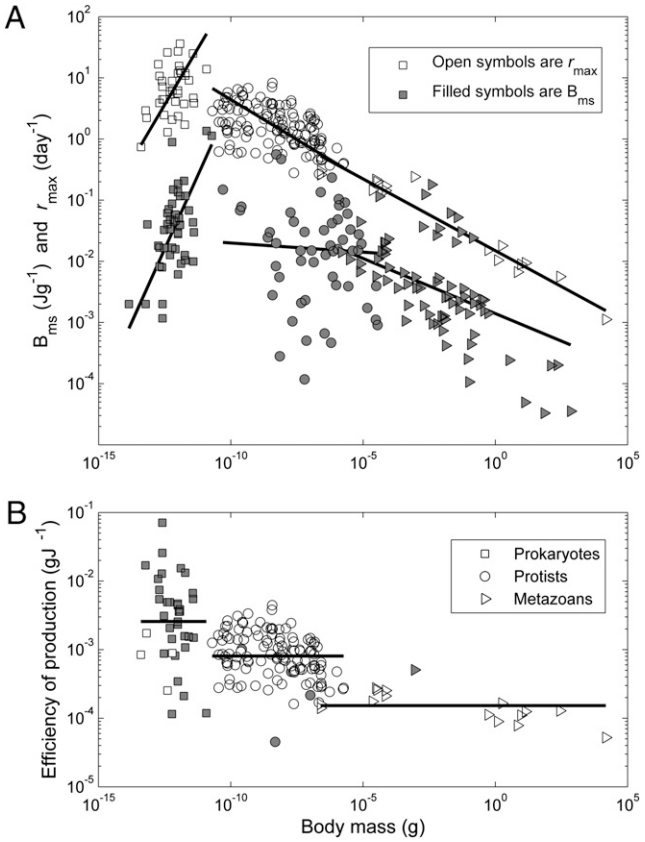

Fig. 2. (*A*) Scaling of r_{max} (unfilled symbols) and mass-specific metabolic rate (B_{ms}, filled symbols) with body mass for heterotrophic prokaryotes, protists, and metazoans plotted on logarithmic axes. For r_{max}, the RMA slopes are 0.73 for prokaryotes, −0.26 for protists, and −0.23 for metazoans. The scalings of r_{max} and B_{ms} within groups are statistically indistinguishable from parallel (*Methods*), consistent with the hypothesis that metabolic rate fuels biomass production. (*B*) Efficiency of biomass production decreases more than tenfold across the three groups, but within a group efficiency is invariant with body size. Closed symbols are for species for which both r_{max} and mass-specific metabolic rates were known. Open symbols are those for which r_{max} values were known for a species but mass-specific metabolic rates were estimated from the regressions in Fig. 1. Horizontal lines represent estimates of the efficiency of production for each group according to the parallel-line regression model for r_{max} and B_{ms} (*Methods*).

prokaryotes to 9.2×10^{-4} for protists and 1.6×10^{-4} for metazoans ($P < 0.001$; Fig. 2*B*). Evidently, the increased whole-organism metabolic rate that accompanies the transitions occurs at the expense of decreased efficiency of conversion of metabolic energy into biomass. The mechanisms underlying this decrease in efficiency with increasing body size and complexity across the transitions warrant investigation. Larger, more complex organisms must allocate relatively more metabolic energy to acquiring and processing food resources and relatively less to biomass production. Some of this decrease may be a result of changes in the organization and location of energy processing machinery. Metabolic processes are extracellular or localized on cell surfaces in prokaryotes, organelle-based in protists, and dependent on complex digestive, respiratory, and circulatory systems in metazoans. So, for example, oxygen is obtained by simple diffusion in unicellular organisms but taken up by gills or lungs and transported through vascular systems in large metazoans. It may not be coincidental, therefore, that each of these evolutionary transitions apparently coincided with major increases in the concentration of oxygen in the atmosphere and oceans (3).

A first step in understanding these transitions is to identify potentially important variables that are associated with the scaling of metabolic rate with body size in each group (Fig. 3). In an initial attempt to account for our unexpected results, we propose the following hypotheses:

Prokaryotes. We hypothesize that the very rapid increase in metabolic rate with increasing cell size is made possible by an increase in the number of genes. If cell size limits the number of genes and/or quantity of DNA, then larger cells can have larger genomes. In prokaryotes, larger genomes have more coding genes, which produce a larger number of different enzymes and result in larger, more complicated biochemical networks. These networks can confer increased metabolic power because cells can use a greater diversity of substrates as energy sources or use a given substrate more completely, thereby producing more ATP molecules per unit substrate and per unit time.

The link between cell size and metabolic network complexity is supported by three empirical findings. First, genome size exhibits the predicted positive scaling with cell size. Fig. 4 shows that both number of genes and total genome size scale with cell size as $M^{0.35}$. The parallel scaling confirms that increasing genome size is a result of increasing numbers of protein-coding genes (21). Second, the proportion of metabolism-related genes increases with genome size in prokaryotes (22). And third, limited data for the five taxa of prokaryotes in the work of Price et al. (23) show a positive scaling relationship between the total number of metabolic reactions and genome size ($R^2 = 0.83$, $y = 12.5x^{0.62}$). These findings are at least consistent with the hypothesis that the superlinear scaling of metabolic rate in prokaryotes is a result of the increase in genome size with body size. Finally, Lauro et al. (24) found all three of these types of mass-dependent effects in bacteria.

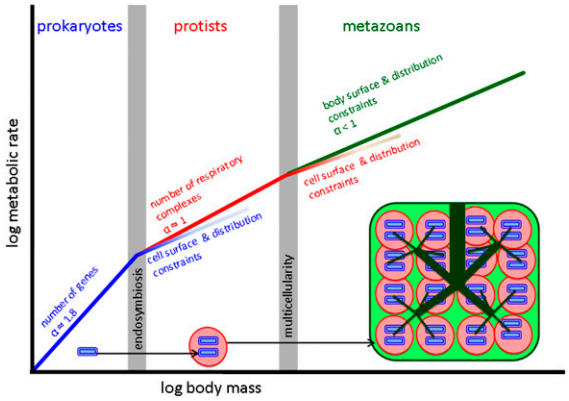

Fig. 3. Schematic representation of our hypotheses to account for the scaling of metabolic rate in prokaryotes, protists, and metazoans. Scaling within each group reflects constraints on metabolic power caused by the number of respiratory complexes, but geometric constraints on exchange surfaces and transport distances ultimately limit capacity to supply substrates to these active sites. Superlinear scaling in prokaryotes (solid blue line) reflects the increase in number of genes and metabolic enzymes with increasing cell size, until a new constraint (fading blue line) resulting from cell surface area, where the enzyme complexes and proton pumps are localized, becomes limiting, imposing sublinear scaling. Protists overcome this constraint because the respiratory complexes are in the mitochondria. Larger protists can accommodate more of these organelles, resulting in linear scaling of metabolic rate with volume of mitochondria and cell mass (solid red line), until a new geometric constraint of surface exchange or transport distance limits rate of resource supply to the mitochondria, imposing sublinear metabolic scaling (fading red line). Metabolic rates of metazoans initially tend to increase linearly with number of cells and body mass, but as vascular systems evolved to distribute resources to increasingly large bodies, geometric constraints required sublinear scaling, converging to the 3/4 power scaling of Kleiber's law (green line).

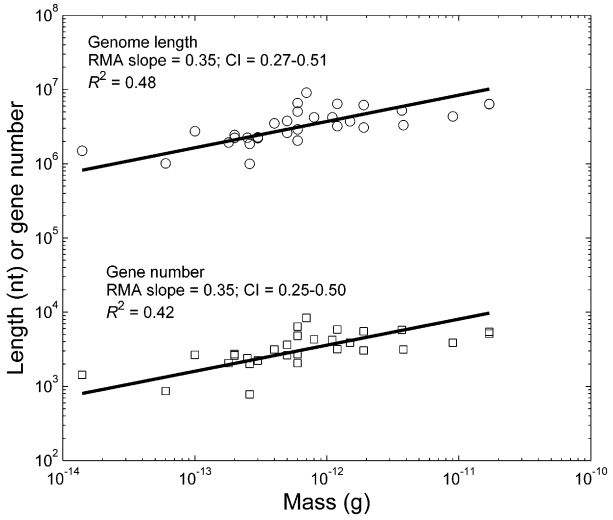

Fig. 4. Scaling of genome size with cell size in prokaryotes. Total number of nucleotides (above) and number of different genes (below) scale with identical slopes of 0.35, consistent with our hypothesis that scaling of metabolic power in prokaryotes reflects the number of genes and the complexity of the biochemical network.

Metabolic power would be expected to increase with increasing genome size only up until the prokaryotic cells have a relatively complete complement of metabolic enzymes and pathways. Indeed, the smallest eukaryotes, such as yeast, have such a complete metabolic network. Moreover, in prokaryotes, the respiratory complexes of enzymes and protein pumps used in ATP synthesis are located in the cell membranes. This would suggest that, with increasing cell size, cell surface area would eventually limit metabolic rate, causing the metabolic scaling to decrease from superlinear toward sublinear. When surface area constraints take hold for prokaryotes, linear scaling in protists allows them to be more powerful and competitively superior to similarly sized bacteria (25), and therefore they begin to dominate at this size. In this way, the superlinear scaling of metabolic rate with mass gives way to linear scaling, at the precise point in size at which bacteria give way to protists along the body size axis.

Protists. We hypothesize that the approximately linear scaling of metabolic rate in protists reflects a linear increase in the membrane-bound sites of ATP synthesis located in organelles. The ancestral heterotrophic eukaryotes were able to overcome the constraints of limited ATP synthesizing sites on the cell surface by ingesting the symbiotic prokaryotes that evolved into mitochondria (26). This innovation allowed the host cell to contain many mitochondria and have a much larger number of respiratory complexes than if the enzymes and proton pumps were located in the external cell membrane. The new design would allow the total volume of respiratory complexes and the metabolic rates of eukaryotic cells to scale linearly with size.

This hypothesis predicts that the number or total volume of mitochondria scales linearly with cell mass, similar to the scaling of organs in metazoans. Support for this hypothesis comes from the linear relationship between mitochondrial volume and cell volume in the alga *Polytoma papillatum* (27) and yeast *Saccharomyces cerevisiae* (28), and the linear relationship between metabolic rate and the volume of mitochondria in cells of metazoans (29, 30).

Such linear scaling cannot be maintained indefinitely, however. As cell volume and number of mitochondria increase, capacity to supply resources to the respiratory complexes eventually becomes limiting, because cell surface area limits the diffusion and number of active sites for uptake of resources from the environment and because distance within the cell limits the diffusion or active transport of materials to the mitochondria. The consequence is a shift from linear to sublinear scaling. At this point, at which surface area constraints take hold for protists, steeper scaling in metazoans allows them to be more powerful and competitively superior to similarly sized protists, and therefore they begin to dominate at this size (25). As with the shift from prokaryotes to protists, the linear scaling of metabolic rate with mass that characterizes protists gives way to sublinear scaling in metazoans, where protists begin to give way to metazoans. Note, however, that there is some overlap in size and metabolic rates of the largest protists and the smallest metazoans.

Metazoans. The next evolutionary transition was the origin of multicellular body plans. Having multiple small cells instead of a single large one allows tiny metazoans to evade constraints of external surface area and internal transport distances. We hypothesize that the scaling in the smallest metazoans is initially near linear, as observed empirically in very small animals and plants (15, 16), at least in the region where there is size overlap between protists and metazoans. As body size increases, however, an increasing fraction of body mass has to be allocated to increasingly differentiated vascular and skeletal systems to provide resource supply and mechanical support. Models of resource distribution through vascular networks show the impossibility of maintaining linear scaling of metabolic rate as body size increases, and several different models independently suggest that the maximal exponent converges to 0.75, or Kleiber's law (14, 31).

Conclusions

The transitions from prokaryotes to unicellular eukaryotes to metazoans allowed many orders of magnitude increase in body size and accompanying diversification of form and function (3). Changes in the scaling of biological energetics over the resultant 16 orders of magnitude in body size reflect the fundamental dependence of metabolic rate on (*i*) the number of membrane-bound respiratory complexes in which proton pumping and ATP synthesis occur and (*ii*) geometric constraints on transport distances and surface exchanges that affect rates of resource supply. Each evolutionary group—prokaryotes, protists, and metazoans—display a distinctive scaling that reflects the particular way in which these two constraints arise. The evolutionary transitions themselves, then, can be seen as giving rise to structural and functional innovations that overcame constraints on their precursors, but imposed new constraints that governed the scaling of metabolic rate. Because metabolism fuels biomass production for growth and reproduction, differences across the transitions in scaling of metabolism are also reflected in transitions in population growth rate and production efficiency.

In conclusion, our data and analyses clearly show that the sublinear metabolic scaling and quarter-power scaling relations documented for large, multicellular animals and plants, with the α values being approximately 0.75 for metabolic rate and −0.25 for r_{max}, do not extend to the smallest organisms. Changes in allometric scaling relations across the major evolutionary transitions identify some of the most fundamental features of biological energetics that shaped the early evolution of life.

Methods

We combined metabolic rate data from several sources, and all data used in these analyses are available in Dataset S1 and Dataset S2. Physiological state has a strong effect on metabolic rates and may influence the observed scaling of metabolic rate with mass (11, 32). We therefore separated data into active and inactive rates. Active rates were defined as rates for which individuals were measured in the presence of food or, if not, in cases in which individuals had been washed free of their food just before measurement. For active and inactive rates of prokaryotes, we used the data sets compiled by Makareiva et al. (17, 18), which are available as supplementary

material attached to their original articles. We only included prokaryote species that are obligate heterotrophs and excluded species capable of phototrophy, chemoautotrophy, and mixotrophy. We also excluded three extremophiles (including the only member of the Archea in the dataset) from the analysis; their inclusion does not significantly change the scaling relation. For inactive rates of protists, we used the data from Makareiva et al. (17), and for inactive rates of small metazoans, we used the zooplankton data from Gillooly et al. (33). For active metabolic rates of protists and zooplankton, we surveyed the literature and developed new data sets. All values in these new data sets were included only after consulting the original references, checking the data, and making sure that the physiological state and other conditions met our criteria for standardization. Multiple values for a species were averaged to create a data set with one mass and one metabolic rate per species. All original metabolic rate units were converted to W, and volumes and masses were converted to grams. The data set for active metabolic rate included 44, 51, and 71 species or strains of prokaryotes, protists, and metazoans, respectively, and for inactive metabolic rate it included 121, 52, and 15 species.

As all data in this study are for ectotherms, temperature strongly influences their metabolic rates. We used the Boltzmann factor with an activation energy of 0.61 eV to correct all data to 20 °C (33). This approach works well because a single correction can be applied to all data, reducing the error variance in the scaling estimates. We analyzed the uncorrected data and still found superlinear scaling in prokaryotes, linear scaling in protists, and sublinear scaling in metazoans, albeit with slightly shallower scaling exponents.

The original studies represented in the data sets used several different methods to measure body mass, including weighing single individuals, and, for unicells, weighing large numbers of cells and dividing by the estimated number of cells and estimating volume from external dimensions. Body mass data were not available in some studies of protists, so we used values from Fenchel and Finlay (11). Differences in the slopes among groups were determined by ANOVA on log-transformed data, comparing models with group-by-slope interaction terms to models without these terms. As indicated earlier, there are several sources of error in the body masses reported in these data sets. The presence of nonnegligible error in the x axis variable means that an ordinary least squares (OLS) fitting procedure is likely to produce scaling slopes that are artificially shallow. Many previous studies on the scaling of unicells have used OLS to estimate exponents, which is one of the many reasons that previous studies on the metabolic rate scaling of protists reported sublinear slopes. As advocated by Smith (34), we correct the slopes and intercepts produced by the ANOVA analysis to the reduced major axis (RMA) equivalent. These corrected parameters are the same as what would be produced by a direct RMA regression, and we present only the corrected slopes. We report SEs from the OLS fitting procedure, which are equal to those produced by RMA (35).

We surveyed the literature to obtain r_{max} values for prokaryotes, and used data from Caron and Rose (36) for protists and from Savage et al. (19) for metazoans. The data set included 37, 122, and 16 species or strains of prokaryotes, protists, and metazoans, respectively. We also collected data for genome size for prokaryotes from the National Center for Biotechnology Information (NCBI) genome database, matching species in our dataset with values for active metabolic rate (18) with species-level data in the NCBI database. For some species, multiple entries, with varying genome sizes, were present in the NCBI database. In these cases, we always used the largest genome size, for consistency.

We estimated the efficiency of biomass production of each species in the r_{max} data set by dividing r_{max} by the active mass-specific metabolic rate. If the active rate was known from the metabolic rate data set, it was used. If the active rate was not known, it was estimated from the regression in Fig. 1. By using ANOVA, we compared a six-slope model (a model allowing for separate slopes and intercepts both between r_{max} and mass-specific metabolic rate B_{ms} and across groups) with a three-slope, parallel-line model (separate intercepts and slopes between groups but the same slopes for r_{max} and B_{ms} within groups) to test whether slopes of B_{ms} and r_{max} differed. Then, because the six-slope model was not significantly better than the three-slope model, we divided the r_{max} intercepts by the B_{ms} intercepts for each group in the three-slope model to get a mean efficiency for each group. With a unit conversion of seconds to days, we expressed efficiency in units of gJ^{-1}.

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