Opinion

Mitochondrial function and cell size – an allometric relationship

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ABSTRACT

Allometric scaling of metabolic rate results in lower total mitochondrial oxygen consumption with increasing organismal size. This is considered as a universal law in biology. Here, we discuss how allometric laws impose size dependent limits to mitochondrial activity at the cellular level. This cell size dependent mitochondrial metabolic activity results in nonlinear scaling of metabolism in proliferating cells, which can explain size homeostasis. The allometry in mitochondrial activity can be controlled through mitochondrial fusion and fission machinery, suggesting that mitochondrial connectivity can bypass transport limitations, the presumed biophysical basis for allometry. As physical size affects cellular functionality, cell size dependent metabolism becomes directly relevant for development, metabolic diseases and aging.

Does cell size matter?

"*Perhaps the most important open question is how size relates to function*" [1]. This statement holds true for all scales of biological organization from organelles and cells to whole organisms and beyond. We argue that function is a key determinant of cell size and that cell size also affects cell function. The human oocyte, one of the largest cell types, is transformed into an extraordinary diversity of different cell types. Each of these cell types is characterised not only by their unique function, but also by their cell size, which is believed to be linked to functionality. While evidence for size dependent functionality is slowly accumulating (see Table 1), not all functions seem to scale similarly with size. Little is known about the mechanisms why a specific size is optimal and what determines this size.

Cell size homeostasis is maintained through cell growth (increase in cell size), cell division and osmotic balance. In most experimental systems the balance between growth and proliferation is the most critical factor for specifying cell size. Size-dependent adjustment of cell cycle length and/or growth rate thus emerges as the main cell size control mechanism in proliferating cells (reviewed in [2]). Because growth rate is proportional to metabolic rate [3], metabolism affects cell size. We will discuss that also the converse is true: metabolism is cell size dependent and this feedback between size and metabolism provides a mechanism for controlling growth and cell size.

Cell surface-to-volume (SV) ratio (see Glossary), intracellular transport distances and diffusion times of oxygen and nutrients are believed to limit metabolism and thus the increase in cell size. While these biophysical mechanisms may explain why cells are small in general [4], they cannot explain why various cell types display their characteristic size, i.e. how target size is determined. Specialization to perform their key function(s) is more likely to explain why different animal cell types can greatly deviate from the biophysically optimal cell size where transport times are minimized and SV ratio maximized. Based on this reasoning, cell size control emerges as a mechanism to ensure appropriate cell physiology and, consequently organismal health, survival and reproduction. However, evidence for this is mostly indirect and correlative: Abnormal cell sizes and increased cell size variability are observed in aging as well as in many common human diseases, including cardiac hypertrophy, type II diabetes, obesity, neurodegenerative diseases and cancer [5]. Coincidentally, these diseases can be classified as metabolic diseases where mitochondrial involvement has been recognized [6].

Mitochondria in cell size control

As a key metabolic organelle, mitochondria control the cellular growth rate. Higher mitochondrial

mass or increased mitochondrial membrane potential translates into higher rate of transcription and translation per unit volume [7-9]. Similarly, our recent data indicates that up to ¾ of cellular variation in translation rate can be explained by mitochondrial activity [10] highlighting the importance of ATP generation through mitochondria for efficient protein synthesis and growth [9, 11]. Some of the metabolic pathways downstream of mitochondria can also decrease cellular catabolism more than they reduce protein synthesis, causing an increase in cell size [12]. While mitochondria are clearly important for setting the overall metabolic activity of the cell, there is some controversy in which way mitochondrial perturbations affect animal cell size [13-15]. Inhibition of oxidative phosphorylation, induction of mitochondrial ROS and mild uncoupling of mitochondrial membrane potential increase cell size and reduce cell proliferation [13]. Yet, at least in yeast, deletions of mitochondrial genes can reduce cell size. Similarly, clinically relevant mutations in mitochondrial DNA can either increase or decrease cell size [15, 16]. Considering that mitochondria are complex organelles with multiple and often interconnected functions ranging from ATP production to catabolism [6], it may not be surprising that different perturbations to mitochondrial homeostasis can induce opposite size phenotypes.

Mitochondria also regulate proliferation through several mechanisms, as cell cycle and mitochondria are closely hardwired [17]. Functional mitochondria are required for proliferation in various model systems [18-20]. In particular, mitochondrial hyperfusion correlates with cyclin E accumulation [21], which is required for cell cycle progression at the G1/S transition. The G1/S transition is critical to the commitment to cell division and a key stage where cell size needs to be monitored [2, 22]. Mitochondrial metabolism is also a main source for cytosolic acetyl-CoA, a metabolite critical for histone acetylation and lipid synthesis. Acetyl-CoA promotes cell proliferation [23] and inhibition of lipid biosynthesis limits proliferation and resulting in larger cell size [12, 13]. Lipid biosynthesis pathways and their transcriptional controllers, including the sterol regulatory element binding proteins (SREBPs), are downregulated at the mRNA level *in vivo* when proliferation is prevented and cells increase in size [13]. Conceptually, these observations are consistent with the reasoning that larger cells have a reduced SV ratio and, consequently reduced need for plasma membrane lipids. It was recently shown that bacterial cell size is controlled by the balanced production of cell surface and volume components [24]. In other words, the SV ratio may impose limits to the maximal cell size and could also be part of a size control strategy. Considering the key role for mitochondria in setting the growth rate through energy generation and affecting cell surface growth through lipid biosynthesis, it should be obvious why mitochondria occupies a crucial position for regulating cell size. It has been proposed that feedback between size and metabolism is an essential

requirement for cell size control [2]. But is there any evidence that mitochondrial activity is cell size dependent?

Cellular allometry, the framework for cell size dependent mitochondrial metabolism

Cellular contents, including mitochondria and other organelles, typically scale isometrically with cell size in growing cells (see Box 1). However, experimental evidence indicates that cultured mammalian cells decrease their growth rate once they have accumulated enough mass [25, 26] and similar observations have been made in phytoplankton [27] and in plants [28]. These findings suggest that some cellular functions must be decoupled from the isometric size-scaling of cellular contents. This is especially true for mitochondria.

For over a hundred years it has been known that larger organisms have a decreased size-normalised metabolic rate, as measured by oxygen consumption [29, 30] (Fig. 1a, top). This phenomenon known as allometric scaling of metabolism (Box 1) is one of the most fundamental features of life and it is believed to apply to all size scales [31], including individual cells [32]. Mitochondria are almost exclusively responsible for cellular oxygen consumption, suggesting that size-normalised mitochondrial activity should be reduced in larger cells (Fig. 1a, bottom). While theoretical models establish the decline in metabolic activity with increasing cell size [32, 33], this has been validated only by comparing different cell types [33]. Our recent study [10] revealed that cellular metabolism scales with cell size also within the same cell population. There is a decline in mitochondrial activity towards larger cell size, as measured using two key mitochondrial parameters, mitochondrial membrane potential and oxidative phosphorylation. Thus, the functional scaling of mitochondria is distinct from the isometric scaling of mitochondrial mass. This metabolic scaling was confirmed in various cell types from different animal species and persists under various cell culture conditions. In addition to body size, metabolic rate is also sensitive to temperature based on biochemical kinetics [34]. We found that the rate at which mitochondrial activity was reduced with increasing cell size also reacted to temperature as predicted by these mathematical models of allometric scaling [34]. Consistently, we have previously observed that mitochondria associated genes display a relative reduction in mRNA levels in response to increases in hepatocyte size *in vivo* [13]. This downregulation of mitochondria-related gene expression likely reflects the reduced demand for mitochondrial function in larger cells. Altogether, the evidence indicates that mitochondrial activity changes with cell size resulting in allometric scaling of metabolism on cellular level.

Metabolic allometry and size homeostasis in dividing cells

In addition to the decline in mitochondrial activity in larger cells within a proliferating cell population, there is a decline in mitochondrial activity in the smallest cells [10]. This cannot be directly explained by allometric scaling laws, but is likely a consequence of the cell cycle. Upon cell division, a large cell with low relative metabolic activity will give rise to two smaller daughter cells, which inherit the mothers' mitochondria proportionally to their size [35, 36]. The inheritance of mitochondrial functionality is very likely linked to the inheritance of mitochondrial content, as has been predicted before [8], causing daughter cells to inherit the low metabolic activity of the mother cell (Fig. 1b). Thus, the newborn cells will start with low mitochondrial activity, which they have to "reset" before allometric scaling of metabolism again starts to limit their metabolic activity. This may explain the observed nonlinear relationship between cell size and mitochondrial activity. While the inheritance of mitochondrial material has been studied extensively, future research should also examine the inheritance of metabolic rate, as predicted here. In proliferating cells, it is also important to remember that specific cell cycle events can affect mitochondrial activity, at least momentarily [17]. However, on a larger size scales, cell size impacts mitochondrial metabolism much more than cell cycle [10], and cells must adjust for this in order to maintain fitness and function.

While cellular allometry cannot be directly expanded to explain differences in mitochondrial activity between various cell types, it can provide a mechanism for a cell population to maintain size homeostasis. By limiting growth in larger cells, allometric metabolism constrains cell size increase. Simultaneously, intrinsic requirements, such as minimal volume requirements, as well as cell type specific functional requirements necessitate larger cell size. The optimal size results from the balance between these size-constraining forces. While more work is needed to understand the biological and biophysical mechanisms imposing cell size limits, allometric scaling of metabolism has the potential to be part of a universal cell size regulator. Furthermore, as metabolic allometry can explain complex biological phenomena, such as population density, lifespan and evolution rate, on an organismal level [31, 37], it seems reasonable to assume that cellular allometry may have profound, although unexplored, biological consequences. One such example is the cellular phenotype seen in aging cells, where mitochondrial activity is reduced [38, 39] and cell size increased, at least in specific cell types [39, 40]. The age-dependent decline in mitochondrial activity could, in theory, be partly due to the underlying cell size increase. Similarly to aging, many diseases, like Alzheimer disease and type II diabetes, which are well-known to display decreased mitochondrial activity, are also characterised by cellular hypertrophy [41-43]. Possible causalities between cell size and mitochondrial activity should be investigated further in these settings.

Mitochondrial fusion and fission link mitochondrial functions to cell size

The rate of metabolic decline with increasing cell size varies between cell types [10, 33] suggesting that cellular functions or structural features affect metabolic scaling. Mitochondria are dynamic organelles, which fuse and divide to control mitochondrial connectivity, energy production, cell proliferation and stress resistance [21, 44, 45]. Our recent work shows that the cell size scaling of mitochondrial functionality is dependent on mitochondrial dynamics. Both genetic and chemical inhibitions of mitochondrial fission promoting Dynamin related protein 1 (DRP1) increase mitochondrial membrane potential and oxidative phosphorylation in larger cells within a population, making mitochondria more active in large, but not in small, cells [10]. Reduced mitochondrial fission also leads to an increase in median cell size of the population. Opposite phenotype is observed when mitochondrial fusion is inhibited by a knockdown of Mitofusin 2 (MFN2). It should be noted that complete knockouts of mitochondrial fusion and fission proteins force cells to adapt to the lack of mitochondrial dynamics, which may result in different outcomes than seen with knockdowns and acute inhibitions. Such a contrast between knockout and knockdown has been seen when examining the effects of DRP1 on cell cycle and growth [46, 47]. In addition to direct perturbations to the mitochondrial fusion and fission machinery, we found that the mevalonate pathway, which regulates both cell size and mitochondrial connectivity, was also capable of altering the cell size scaling of mitochondrial activity [10, 12]. However, the best known growth and cell size regulating pathway, the mTOR pathway, did not affect the cell size dependent mitochondrial metabolism [10].

The allometric scaling of cellular metabolism and its dependence on mitochondrial connectivity could, in theory, be explained by proton leakage. Proton leakage has been shown to be partly dependent on mitochondrial inner membrane area [48], suggesting that highly connected mitochondria would have less leakage. However, a recent study in *C. elegans* has reported opposite effects, as proton leakage was reduced by deletion of mitochondrial fusion proteins [49], suggesting that proton leakage is low in highly fragmented mitochondria. Furthermore, we have not observed strong nonlinear proton leakage under unperturbed conditions and inhibition of DRP1 actually increases leakage in the larger cells [10]. Thus, proton leakage is an unlikely explanation for the nonlinear cellular metabolism and its control through mitochondrial dynamics.

Mitochondrial dynamics enables controllable allometric scaling

Biologists have long speculated that mitochondrial structure may affect the allometric scaling of metabolism [48, 50]. The observation that mitochondrial dynamics/connectivity controls the cell size

scaling of mitochondrial activity suggests that allometric scaling of metabolism is under active control within each cell and tissue, as mitochondrial network connectivity can react to both intrinsic and extrinsic cues [51]. This active and cell type dependent control of mitochondrial dynamics may help explain cell type specific allometric scaling patterns [10, 33], organismal activity dependent allometric scaling patterns [37], and the fundamental difference in allometric scaling between eukaryotes and prokaryotes [52].

Changes in mitochondrial dynamics could in principle allow deviations from the ¾ power law of allometry [30, 32, 34, 37, 53] although the mechanistic basis for this remains unclear. One of the widely-accepted causes for allometric metabolism is transport limitations and maximal cell size is thought to be constrained by the same reason [4]. The metabolic theory of ecology [53] has gained popularity in explaining organismal, population and ecosystem level processes. This general theory on allometric scaling of metabolism suggests that natural selection has created hierarchical fractal-like transportation networks on all size-scales of life and that allometric scaling is a result of transportation limits in these networks. Such fractal-like transportation networks have been optimised for maximizing the surface areas for best metabolic capacity and by minimizing the transport distances and times [54]. Curiously, mitochondria also form fractal-like networks, where the network structure may be crucial for allometric scaling of metabolism.

Metabolites and oxygen will have to diffuse from cell surface to the mitochondria before they can be used for oxidative phosphorylation. Small cells have short intracellular distances, which makes metabolism in small cells less affected by transportation distances than large cells (Fig. 2, left and middle). As described above, increased mitochondrial connectivity increases mitochondrial activity in large cells, suggesting that mitochondrial connectivity can somehow overcome transport limitations. Therefore, mitochondrial networks must act as transportation systems for the limiting metabolite or molecule. This transportation through fused mitochondria should also be faster than diffusion, on which large cells with highly fragmented mitochondria would rely on. We hypothesise that the transport-limited, and also allometry-inducing, factor is mitochondrial energy supply itself. Mitochondria have been proposed to act as energy conducting routes already several decades ago (see [55] for a review). Membrane potential can propagate within the mitochondria much faster than diffusion of particles [56, 57], supporting the hypothesis that mitochondrial reticulum transports energy in the form of proton motive force. Therefore, mitochondria close to the cell surface, where metabolite and/or oxygen levels are highest, can generate the proton motive force that is transported and used in a separate part of the mitochondrial network in the inner parts of the cell (Fig. 2, right). Such intracellular ‘electrical cabling’ would enable highly efficient spread of energy to all parts of

the cell to which the same mitochondrial network extends. Therefore, mitochondria would have to be not only highly connected, but also distributed so that one part of the mitochondrial network is close to cell surface whereas other part of the mitochondrial network is next to the location where energy is most needed, like the nucleus or in the cytoplasmic parts with abundant ribosomes.

Our hypothesis, which suggests that mitochondrial networks support larger cell size through energy transportation, makes several predictions. First, it predicts that the molecules required for energy production should display a gradient towards the centre of the cell. While more quantitative experiments are needed, current experimental evidence suggests that at least oxygen levels are higher close to cell surface and lower in the middle of cells [58]. Second, the size-dependent decline in mitochondrial metabolism should be more pronounced in conditions where cells are highly dependent on mitochondria for energy production. We have shown this by culturing cells in galactose instead of glucose [10]. Third, high mitochondrial connectivity would only be needed in conditions, where limited nutrient availability forces cells to utilize oxidative phosphorylation for energy production. Consistently, mitochondria are well-known to fragment under high nutrient conditions [59], whereas in conditions where cells are dependent on oxidative phosphorylation mitochondrial fission is reduced [60]. Fourth, high mitochondrial connectivity should allow larger cell size, as we have seen in our experiments. This is also consistent with evolutionary logic suggesting that mitochondria enabled the large eukaryotic cell size [61]. Fifth, complex (nonspherical) cell shapes, where intracellular distances from plasma membrane to the centre of the cell are maintained short, should be beneficial for large cells. This holds true for most cell types in the human body. The smallest cells, like lymphocytes, are typically very spherical, whereas the largest cell types, like neurons and skeletal muscle cells, have an extremely elongated and often branching morphology. Our hypothesis that mitochondrial networks act as transportation system to enable larger cell size could be further tested by, for example, carefully examining mitochondrial membrane potential in mitochondria localized to different parts of the cell. Mitochondria close to the centre of the cell should have reduced membrane potential, unless they are connected to mitochondria close to the cell surface.

Concluding Remarks

It is becoming increasingly evident that many cellular activities are sensitive to or even regulated by cell size (Table 1). All these findings are indicative of the presence of an optimal cell size that reflects optimal cellular function. As a consequence, understanding the mechanisms that control cell size is fundamental for understanding cell physiology.

It is easy to visualise that mitochondrial activity is important for setting the overall metabolic activity, growth, proliferation and, consequently cell size. Disentangling how various mitochondrial functions affect growth and proliferation will be critical for a more complete understanding of cell size control. The cell size dependent metabolism is consistent with allometric scaling of metabolism and provides proliferating cells with the nonlinear functionality critical for cell size homeostasis. Allometric scaling has been suggested to be partly dependent on cell proliferation [33], and cell size control based on metabolism could thus differ between proliferating and non-proliferating cells. Regardless, the universal nature of allometric scaling of metabolism appears to extend to the cellular level with broad implications for cell size regulation, fitness and cellular metabolism in general.

The cell size dependent metabolism raises several questions with potential major implications for both basic biology and biomedical research (see Outstanding questions). For example, apart from mitochondrial activity, what metabolic processes or other cellular functions depend on size? If intracellular distances and/or SV ratio limit cell size, do more elaborate cell shapes enable higher mitochondrial activity and larger cell size? And most importantly, as cell size can influence metabolism and growth, is a wrong cell size necessary or even sufficient for developing metabolic pathologies? Cell size should be more closely investigated in the context of development, aging and disease, as better understanding of size dependent metabolism could provide new treatment options for diseases where cell size has changed.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

BOX 1. Cellular allometry

Allometry is the study of biological scaling in comparison to size. Classically, allometry has focused on the relationship between metabolic rate and body size in animals, although it also extends to plants and single-cell organisms. Allometry has been extensively studied by comparing oxygen consumption rates, which is a proxy for overall metabolic rate, between different sized organisms. It is now widely accepted that larger organisms have reduced metabolic rate in comparison to their size

(Fig. 1a), as metabolic rate typically scales according to the ¾ power law (see Glossary) [31, 37]. This is generally viewed as a consequence of size-dependent limitations in nutrient and/or oxygen transport, which cause metabolic rate to decrease in larger animals. However, size-dependent increase in metabolic efficiency cannot be fully discarded as an explanation.

At the cellular level, most studies have focused on the size scaling of cellular content. It is now clear that cellular organelle and protein content increases isometrically (linearly) across a wide range of cell sizes. This observation applies to multiple organelles, including nucleus [62, 63], mitochondria [36, 64] and spindle [65, 66]. Much less is known about how organelle functionality or cell metabolism changes with cell size.

Theoretical analyses have indicated that allometric scaling of metabolism applies to cellular level [32], but experimental evidence has remained limited as most studies examining cell size dependent metabolism analysed interspecies differences. In addition, while allometric laws predict a decline in metabolic rate with increasing cell mass, such decline occurs relative to the unit volume, a fact often ignored in many cellular studies.

Glossary

- **¾ power law:** A widely applicable observation that organismal metabolic rate (R) is proportional to the mass (M) as follows: $R \propto M^{3/4}$. Also known as Kleiber's law.
- **Allometric scaling:** Scaling where the measured parameter changes at a different rate compared to the size of the organism (or cell). For example, if growth rate (G) scales allometrically with organism mass (M) then: $G \propto M^{B}$, where B is a scaling exponent and $B \neq 1$
- **Isometric scaling:** The measured parameter (e.g., growth, function) equals the increase in size of the organism (or cell). For example, if growth rate (G) scales isometrically with organism mass (M) then mathematically, G is directly proportional to M ($G \propto M$).
- **Mitochondrial dynamics:** The process of mitochondrial fusion and fission, which is responsible for the shaping of the mitochondrial network.
- **Mitochondrial connectivity:** The degree to which mitochondria within each cell are connected to each other through mitochondrial fusion. In an extreme case, mitochondria can fuse in to a complex, unfragmented network where individual mitochondria cannot be distinguished.
- **SV ratio:** Surface-to-volume ratio

Figure legends

Figure 1. Allometric scaling of metabolism on a cellular level. (a) The principle of allometric scaling of metabolism. *Top,* The metabolic rate of organisms is inversely correlated with their mass. *Bottom,* allometric scaling of metabolism is predicted to apply to the cellular level so that larger cells will have lower metabolic rate in comparison to their size. **(b)** Allometric scaling of metabolism in proliferating cells. Increase in cell size during the cell cycle will reduce mitochondrial activity, as predicted by allometric scaling of metabolism. However, the scaling pattern of proliferating cells is not linear. This is likely due to the fact that the newborn daughter cells will inherit their mother's low metabolic rate and, as the cell daughters start to grow, they will reset their mitochondrial activity to match their actual size.

Figure 2. Potential mechanism for mitochondrial connectivity in controlling the allometric scaling of metabolism. Small cells will not be limited by large intracellular transport distances and can therefore maintain a higher relative metabolic rate. As cells grow larger their intracellular distances will increase and this will impose metabolic limitations for the cells, as transport of metabolites and oxygen becomes limiting. However, high mitochondrial connectivity can overcome these size-dependent metabolic limitations. This can be explained by the fact that mitochondrial networks can act as intracellular 'power cables' transporting proton motive force through the mitochondrial network much faster than metabolites can diffuse. Thus, in order to provide adequate energy throughout the cell to maintain high metabolic rate, nutrients and oxygen will only need to diffuse to the mitochondria closest to the cell surface, where proton motive force can be generated and then passed on to the rest of the mitochondrial network. However, a hyperfused mitochondrial network may interfere with cell division and limit mitophagy, among other things, thus explaining why cells do not maintain constantly hyperfused mitochondrial networks [67, 68].

Table 1. Animal cell size dependent functionalities.

Organism(s)	Cell type(s)	Measured function(s)	Correlation with cell size	Reference
Homo sapiens	Fibroblasts	Proliferative capacity	Nonlinear, intermediate sized cells proliferate most	[69]
Homo sapiens	Corneal epithelial cells	Proliferative capacity, stemness	Negative, linearity unclear	[70]
Rattus norvegicus	Adipocytes	Lipid metabolism	Larger cells have more active lipid metabolism	[71]
Caenorhabditis elegans	Early embryos	Spindled assembly checkpoint (SAC) strength	Nonlinear, smallest cells have highest SAC strength	[72]
Rattus norvegicus	Pancreatic β cells	Insulin secretion	Linear, positive	[73]
Caenorhabditis elegans	Early embryos	Spindle elongation speed	Linear, positive	[74]
Homo sapiens, Rattus norvegicus, Gallus gallus, Drosophila melanogaster	Various	Mitochondrial activity, proliferative capacity, cellular fitness	Nonlinear, intermediate sized cells have highest functionality	[10]
Rattus norvegicus	Skin keratinocytes	Proliferative capacity	Nonlinear, intermediate sized cells proliferate most	[75]
Mus musculus	Lymphoblasts and pro-B-cell lymphoids	Growth rate	Nonlinear, intermediate sized cells have highest growth rate	[25, 26]

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Figure 1

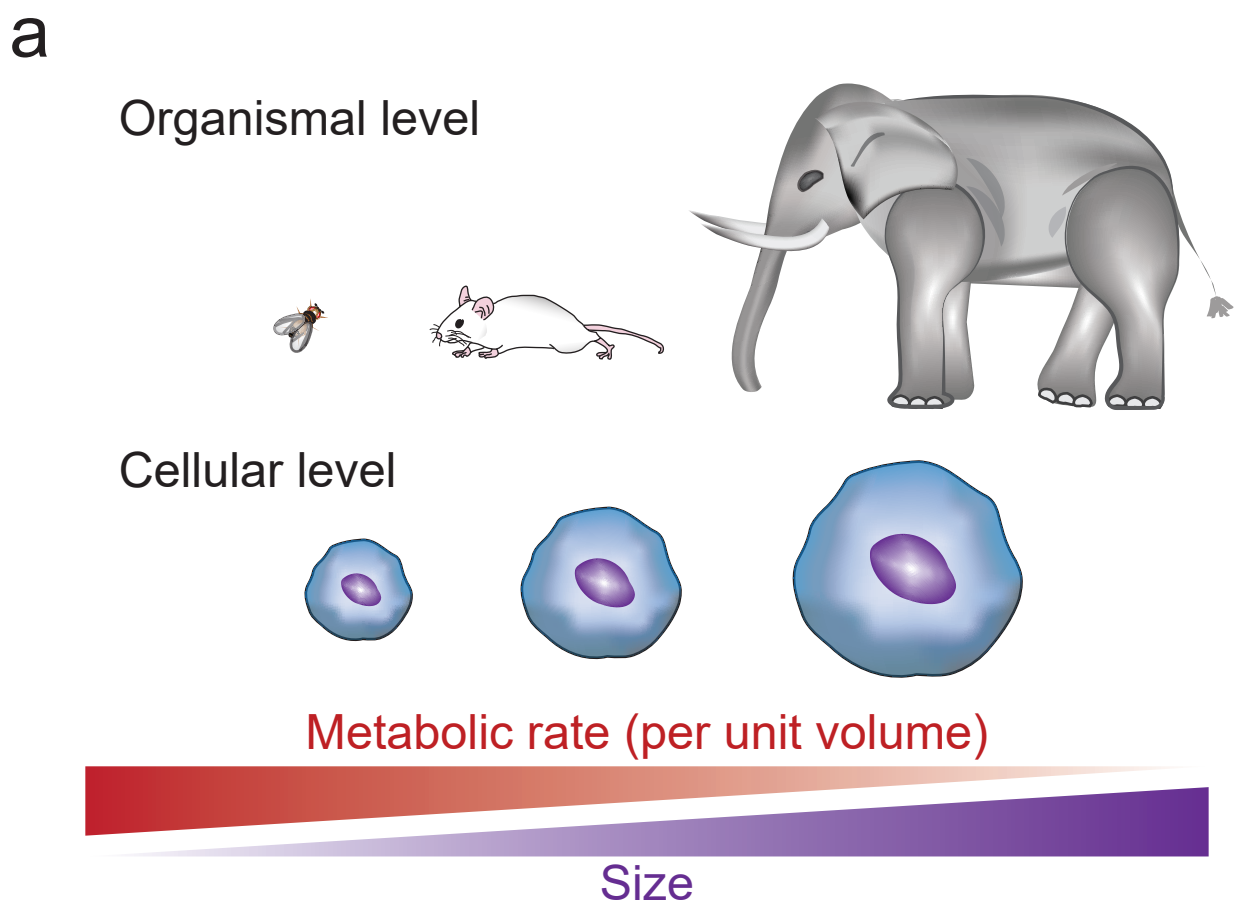
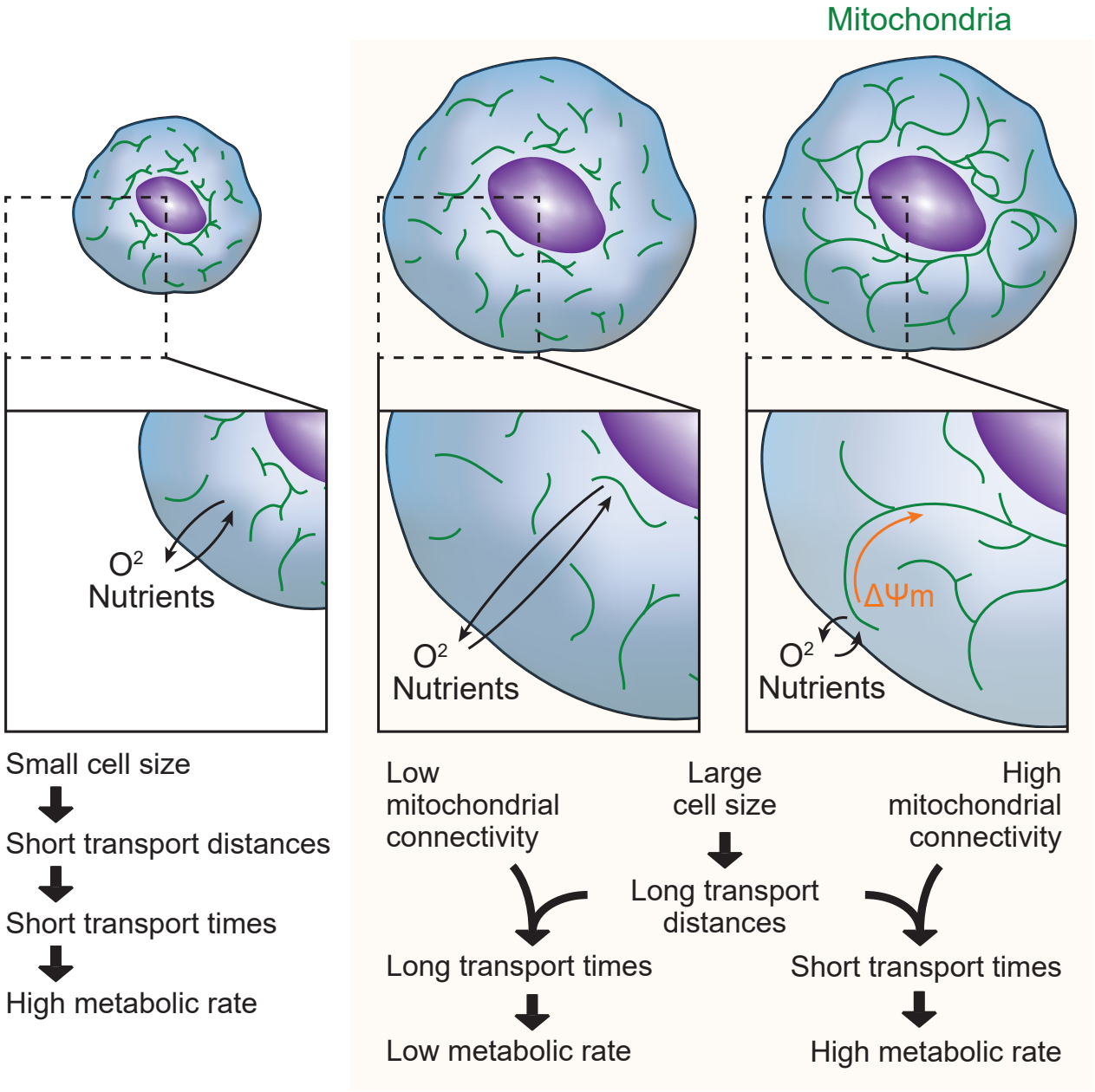

a
Organismal level
Cellular level
Metabolic rate (per unit volume)
Size
b
Mitochondrial activity
Cell division
Metabolic status inherited
Metabolic reset
Allometric scaling of metabolism
G1
S
G2
M
Time
Cell size

Figure 2


Mitochondria
O2
Nutrients
O2
Nutrients
ΔΨm
O2
Nutrients
Small cell size
Short transport distances
Short transport times
High metabolic rate
Low mitochondrial connectivity
Large cell size
High mitochondrial connectivity
Long transport distances
Long transport times
Short transport times
Low metabolic rate
High metabolic rate