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Scaling of biosynthesis and metabolism with cell size

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ABSTRACT **Cells adopt a size that is optimal for their function, and pushing them beyond this limit can cause cell aging and death by senescence or reduce proliferative potential. However, by increasing their genome copy number (ploidy), cells can increase their size dramatically and homeostatically maintain physiological properties such as biosynthesis rate. Recent studies investigating the relationship between cell size and rates of biosynthesis and metabolism under normal, polyploid, and pathological conditions are revealing new insights into how cells attain the best function or fitness for their size by tuning processes including transcription, translation, and mitochondrial respiration. A new frontier is to connect single-cell scaling relationships with tissue and whole-organism physiology, which promises to reveal molecular and evolutionary principles underlying the astonishing diversity of size observed across the tree of life.**

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INTRODUCTION

Cell size is determined by the relative rates of cell growth and division, and in the past two decades, impressive progress has been made in understanding different mechanisms of size regulation (Campos *et al.*, 2014; Pan *et al.*, 2014; Adiciptaningrum *et al.*, 2015; Ho and Amir, 2015; Schmoller *et al.*, 2015; Taheri-Araghi *et al.*, 2015; Harris and Theriot, 2016; Varsano *et al.*. 2017; Cadart *et al.*, 2018; Garmendia-Torres *et al.*, 2018; Ginzberg *et al.*, 2018; Liu *et al.*, 2018; Micali *et al.*, 2018a,b; Facchetti *et al.*, 2019; Barber *et al.*, 2020; Xie and Skotheim, 2020; Zatulovskiy *et al.*, 2020; Tan *et al.*, 2021). For example, unlike yeast and bacteria, animal cells modulate growth rate in addition to cell cycle duration in order to maintain size homeostasis (Kafri *et al.*, 2013; Cadart *et al.*, 2018; Ginzberg *et al.*, 2018). Further complexity has emerged as different size parameters including mass, volume, and protein levels appear to be regulated by distinct pathways (Delarue *et al.*, 2018; Demian *et al.*, 2019; Knapp *et al.*, 2019) whose uncoupling can be pathological. For example, work in budding yeast revealed the existence of a cell size threshold beyond which biosynthesis does not scale with cell volume, causing dilution of cell contents and premature senescence (Neurohr *et al.*, 2019). In contrast, fission yeast cells display variations in density during the cell cycle under physiological conditions, indicating that variation in intracellular concentrations can also occur normally (Odermatt *et al.*, 2021). In mammals, enlarged cell size is a feature of aging cells (Biran *et al.*, 2017) and was shown to reduce stem cell potential in mice (Lengefeld *et al.*, 2021), providing further evidence that size regulation is important for proper cell function. These findings highlight a fundamental question: how do cellular physiological processes such as biosynthesis or metabolism scale with cell size? Previous reviews have emphasized mechanisms of cell size control (Willis and Huang, 2017; Schmoller, 2017; Ho *et al.*, 2018; Jonas *et al.*, 2018; Zatulovskiy and Skotheim, 2020), the coupling of growth with different size parameters (Cadart *et al.*, 2019; Xie *et al.*, 2022), and the scaling of organelles with cell size (Levy and Heald, 2016; Miller *et al.*, 2020). This review focuses on scaling of biosynthesis and metabolism with cell size and the consequences of disrupting this scaling. For multicellular organisms, size-dependent relationships between cellular physiology and function at the level of an organ or whole organism are poorly understood but have important implications in areas as diverse as ecology (Liedtke *et al.*, 2018) and cancer (Schoenfelder and Fox, 2015).

TRANSCRIPTION, TRANSLATION, AND GROWTH RATE INCREASE WITH CELL SIZE BUT SHOW DISTINCT PATTERNS

At the most basic level, maintaining intracellular homeostasis during cell growth requires that a cell produce its components in the correct amounts, thereby maintaining macromolecular concentrations

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Abbreviations used: ATP, adenosine triphosphate; DNA, deoxyribonucleic acid; HSCs, hematopoietic stem cells; RNA, ribonucleic acid; RPE1, retinal pigment epithelial cells; SILAC, stable isotope labeling by amino acids in cell culture.

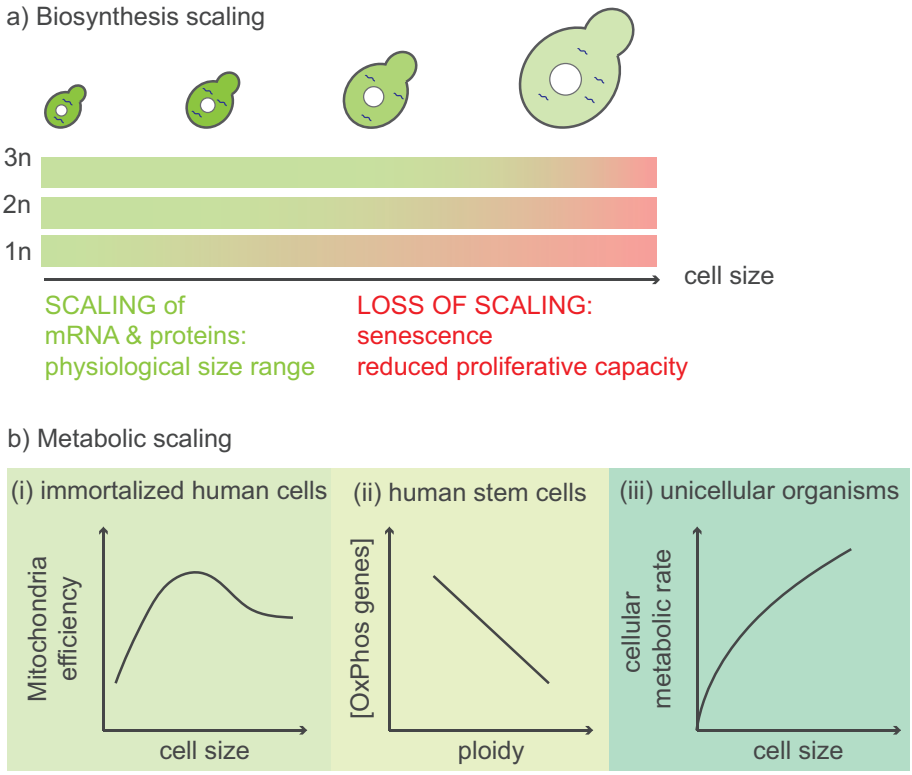

FIGURE 1: Examples of biosynthesis and metabolism scaling with cell size. (a) In budding yeast, mRNA and protein levels scale with cell size. The upper limit to a physiologically fit size range is thought to be set by ploidy, which can become rate-limiting for transcription. (b) Cellular respiration and metabolic rate appear to decrease with cell size. (i) In immortalized human cells, mitochondrial efficiency is optimal at intermediate cell sizes (Miettinen and Bjorklund, 2016). (ii) In human stem cells, mitochondrial- and nuclear-encoded oxidative phosphorylation genes are expressed at higher levels in haploids compared with diploids (Sagi *et al.*, 2016). (iii) Across unicellular organisms, a power law with an exponent <1 relates cellular metabolic rate and cell size (Glazier, 2009).

as cell size increases (Schmoller and Skotheim, 2015). Many cell types grow exponentially in vitro (Cadart *et al.* 2019), dictating that synthesis rates scale accordingly. *Escherichia coli* provides the simplest case, with cells growing at a constant exponential rate that scales both with cell size (Si *et al.*, 2017) and with the protein synthesis machinery (ribosome mass fraction) (Scott *et al.*, 2010). The growth rate of budding yeast cells also correlates directly with the ribosome mass fraction (Kafri *et al.*, 2016) and is thought to be exponential (Talia *et al.*, 2007) but other studies have suggested that growth rate may vary across the cell cycle (Ferrezuelo *et al.*, 2012). Growth of *Schizosaccharomyces pombe* was recently shown to be exponential (Pickering *et al.*, 2019) over a large range of sizes (including mutants that were up to fivefold the size of wild-type cells [Knapp *et al.*, 2019]), contradicting a long-standing belief that these cells follow a bilinear mode of growth (Horváth *et al.*, 2013). New techniques allowing high-throughput single-cell growth measurement in animal cells have revealed that they deviate more dramatically from monoexponential growth and show complex mass (Mu *et al.*, 2020) and volume (Cadart *et al.*, 2018) fluctuations, with a growth rate that is 15% higher in S-G2 than in G1 (Cadart *et al.*, 2022). The origin of cell cycle–dependent changes in growth rate is mysterious and cannot be explained by variation in transcription rate, because transcript amounts scale linearly with cell size (Padovan-Merhar *et al.*, 2015; Sun *et al.*, 2020; Swaffer *et al.*, 2021), independent of genome doubling during S phase. Across diverse organisms, relationships between cell size and growth are still being characterized with the help of newer and more accurate single-cell measurement methods. In many cases, cell size, transcription, translation and volume growth rates show distinct patterns across the cell cycle. Fundamentally, the mechanisms that coordinate biosynthetic processes remain poorly understood, but studies in budding yeast are providing new insight, with recent studies demonstrating that the linear scaling between cell size and transcript levels involved regulation of both RNA polymerase II (Sun *et al.*, 2020; Swaffer *et al.*, 2021) and mRNA stability (Swaffer *et al.*, 2021).

INCREASING PLOIDY EXTENDS THE RANGE IN WHICH BIOSYNTHESIS SCALES WITH CELL SIZE

In a landmark study, Neurhor and colleagues analyzed the consequences of extreme cell size in budding yeast by transiently blocking cell cycle progression to obtain haploid cells that were up to sixfold larger than normal (Neurohr *et al.*, 2019). Volume growth of large cells outpaced protein and RNA synthesis rates, leading to cytoplasm dilution (Neurohr *et al.*, 2019). Importantly, uncoupling of volume growth rate and protein synthesis occurred at a larger cell size in diploids, indicating that transcripts become rate-limiting for translation (Lin and Amir, 2018; Metzl-Raz *et al.*, 2020) and that the ploidy sets an upper bound to the range in which biosynthesis scales with cell size (Figure 1a). Polyploidy has long been hypothesized as a mechanism to increase the metabolic capacity of highly synthetically active cells such as *Drosophila* nurse cells or salivary gland cells (Frawley and Orr-Weaver, 2015) by providing more gene copies and thus increasing rates of biosynthesis. However, measurements of cultured animal cells in suspension spanning ploidies from 2N to 64N revealed a constant mass-normalized growth rate that did not increase with genome copy number (Mu *et al.*, 2020). Interestingly, because these cells were in suspension and had a spherical shape, these findings refute the hypothesis that nutrient import, which is dictated by surface area and scales quadratically with volume for a sphere, is rate-limiting and slows biosynthesis, because cells with volumes three orders of magnitude larger than normal displayed similar growth rates. Thus, increased ploidy boosts the ability of cells to obtain larger sizes with constant cytoplasmic density by maintaining a constant size-normalized biosynthesis rate.

The connection between genome size and cell size has long been recognized, though its basis is not understood. Ploidy and cell volume have been shown to scale linearly in budding yeast (Jorgensen *et al.*, 2007; Yahya *et al.*, 2021) and fission yeast (Neumann and Nurse, 2007). Importantly, the correlation between ploidy and cell size within a species (Gillooly *et al.*, 2015) differs from the scaling relationship between genome size and average erythrocyte size observed across species (Gregory, 2001), suggesting that chromosomal mass alone does not completely account for effects of ploidy on cell size, with mechanisms still currently debated (Gregory, 2001;

Cantwell and Dey, 2021). The few available quantifications in animals seem to tell a story different from that in unicellular organisms because, for example, the volume of muscle fibers in vivo scales sublinearly with ploidy in adult humans and adult and developing mice (Cramer *et al.,* 2020; Hansson *et al.,* 2020). Different cell types in humans also display a sublinear scaling relationship between average cell size and ploidy (Gillooly *et al.*, 2015). It is therefore possible that other factors limit the increase in cell size enabled by polyploidization. Conversely, a recent study suggests that a minimal cell size is required to sustain polyploidy. Gemble and colleagues showed that cultured RPE1 cells induced to undergo whole-genome duplication in a single cell cycle grew a similar extent during the following G1 phase compared with their diploid counterparts (Gemble *et al.,* 2022). The newborn tetraploids then accumulated high rates of DNA damage during the subsequent S phase, a phenomenon that was rescued if tetraploid cells were induced to spend a longer time growing in G1. Thus, proteome scaling with ploidy, including all the factors necessary for DNA replication, is necessary for cells to remain healthy while undergoing polyploidization. To date, the mechanisms leading to an increase in cell size following polyploidization remain largely unknown (Frawley and Orr-Weaver, 2015) and it is becoming increasingly clear that the proper coordination of ploidy, cell volume, and mass has important implications for cell physiology.

INCREASED CELL SIZE IS ASSOCIATED WITH BIOSYNTHESIS SCALING DEFECTS AND CAN LEAD TO SENESCENCE

Several recent studies provided evidence that increases in cell size are not a consequence, but a cause, of senescence. Budding yeast cells induced to reach large sizes with more dilute cytoplasm showed physiological defects leading to stress pathway activation, decreased proliferation rate, and premature senescence (Neurohr *et al.,* 2019). In an elegant study, Lengefeld and colleagues brought further evidence for a causal link between cell size and senescence in mouse hematopoietic stem cells (HSCs) in vivo (Lengefeld *et al.,* 2021). In mice, radiation treatment caused HSCs to enlarge and become senescent, a phenotype that could be rescued by treatment with rapamycin to maintain their size small. Conversely, inhibition of cell cycle proliferation caused up to a 15% increase in cell size, led to DNA damage, and impaired the ability of treated HSCs to reconstitute the hematopoietic lineage in recipient mice in a manner that depended on cell size.

The mechanisms causing senescence when cell size increases remain unclear, but a defect in proteome scaling with cell size has emerged as an important factor. A novel triple-SILAC approach to analyze subcellular compartment-specific scaling of the proteome with cell size revealed that in cultured human cells, components typically associated with cell senescence such as lysosomes, β-galactosidase, and metalloproteases were up-regulated with enlarged cell size (Lanz *et al.,* 2021). Thus, contrary to the simple view that all proteins and organelles adapt in the same way to cell size (Levy and Heald, 2012), several processes appear to deviate from a linear scaling pattern (Cheng *et al.,* 2021; Lanz *et al.,* 2021; Liu *et al.,* 2021). Furthermore, when cells reach sizes beyond their physiological range, overall proteome content no longer scales with size and the cytoplasm becomes diluted, presumably due to defective coordination of cell volume growth and biosynthesis. The consequences of cytoplasm dilution are an active area of research, with recent evidence demonstrating effects on reaction rates (Jin *et al.,* 2022; Molines *et al.,* 2022) and phase separation (Delarue *et al.,* 2018) that could negatively impact cell function. New techniques enabling cellular density measurement with unprecedented precision (Miettinen *et al.,* 2022; Oh *et al.,* 2022), as well as theoretical approaches applying principles of colloidal physics to study cytoplasmic crowding (Maheshwari *et al.,* 2019), are likely to provide exciting new insights.

OXIDATIVE PHOSPHORYLATION IS THOUGHT TO CHANGE WITH CELL SIZE AND PLOIDY

While many studies have focused on the importance of cytoplasmic density and scaling of biosynthesis for cell viability (Neurohr and Amon, 2020), characterization of enlarged senescent mouse HSCs also revealed metabolic defects, including a decrease in mitochondrial concentration and lower levels of reactive oxygen species (Lengefeld *et al.,* 2021). Although the metabolic consequences of enlarged cell size remain poorly understood, one important set of results was provided by Miettinen and colleagues, who showed that hepatocytes remodeled their metabolome as their size increased, decreasing mitochondrial oxidative phosphorylation (Miettinen *et al.,* 2014). They also reported that although the concentration of mitochondria remained constant over a range of cell sizes, mitochondrial membrane potential reached a peak at an intermediate cell size (Miettinen and Bjorklund, 2016) (Figure 1b). For a review on the relationship between mitochondria and cell size, see Miettinen and Björklund (2017). Reinforcing the idea that oxidative phosphorylation decreases with increasing cell size, studies of polyploid yeast revealed down-regulation of several proteins involved in mitochondrial respiration (Yahya *et al.,* 2021). Furthermore, in human embryonic stem cells, expression levels of both mitochondrial and nuclear genes involved in mitochondrial respiration were up-regulated in haploid cells (Sagi *et al.,* 2016) (Figure 1b). New approaches to quantify ATP production (Yaginuma and Okada, 2021) and mitochondrial function at the single-cell level (Papagiannakis *et al.,* 2017; Kang *et al.,* 2020) will help elucidate connections between expression of mitochondrial proteins and cellular respiration rate.

SCALING OF CELLULAR RESPIRATION AND METABOLIC RATE WITH CELL SIZE

Why should cellular respiration decrease with cell size? Since the famous ¾ power law between body size and metabolic rate initially observed in animals by Kleiber was extended to unicellular organisms (Savage *et al.,* 2004; Glazier, 2009), cellular metabolic rate was assumed to scale sublinearly with cell size across species (Figure 1b). However, in multicellular organisms, cell type and whole-organism metabolism impact this relationship and add a layer of complexity, which may lead to a trade-off between average cell size and cellular metabolic rate. Highly proliferative and biosynthetically active cells such as epithelial cells maintain a constant size and appear to scale their metabolic rate to that of the whole body. In contrast, other cells maintain a more constant metabolic rate while their size tends to increase with body size—storage cells like adipocytes seem to fall in this category (Savage *et al.,* 2007). Quantitative experiments in planarians recently provided evidence that cell size, body size, and body metabolic rate are connected and showed that a nonlinear increase in average cell size, not cellular metabolic rate, accounted for the conservation of Kleiber's law as body size increased over several orders of magnitude (Thommen *et al.,* 2019). However, while cross-species studies have established that scaling between cellular metabolic rate and cell size occurs, we lack a clear understanding of this relationship, and direct quantification of in vivo cellular respiration in multicellular animals is needed to bring more conclusive answers.

Novel investigations of cellular bioenergetics (Yang *et al.,* 2021) provide a promising approach to understanding how cellular

metabolism changes with cell size by helping identify 1) the key energy-producing components that could be affected by changes in cellular geometry and 2) the energetic cost of central cellular processes whose relative usage could change with cell size or ploidy. One example in the first category is the proposition that the decrease in surface area to volume ratio that occurs as *E. coli* cells grow larger leads to saturation of membrane space available for respiratory proteins, triggering activation of fermentation pathways (Zhuang *et al.*, 2011; Szenk *et al.*, 2017). Reminiscent of this idea, in animal cells, it has been proposed that changes in mitochondrial surface area-to-volume ratio and activity could emerge with changes in cell size due to network remodeling, although scant experimental evidence supports this hypothesis (Miettinen and Björklund, 2017). Moreover, how changes in mitochondrial morphology might relate to decreased expression of mitochondrial genes observed with increasing ploidy in animal cells and budding yeast (Sagi *et al.*, 2016; Yahya *et al.*, 2021) is unclear. Is it possible that overall cellular energy demand changes with cell ploidy or size? The development of approaches deconvolving the energetic cost of key cellular processes, including nutrient import, membrane synthesis, energy production, and each step of the central dogma, similarly to what has been done in *E. coli* (Belliveau *et al.*, 2021), will likely bring insights. In zebrafish embryos, for example, energy expenditure scales with cellular plasma membrane synthesis (Rodenfels *et al.*, 2020), not cell volume, thus demonstrating that cell number, size, and geometry relate to whole-organism metabolism.

CONCLUSION

Recent studies provide compelling evidence that enlarged cell size decreases the ability to thrive (fitness) by contributing to cellular senescence (Neurohr *et al.*, 2019; Lanz *et al.*, 2021) and loss of replicative potential (Lengefeld *et al.*, 2021). The emerging view is that defective scaling between biosynthesis and cell size, which is limited by ploidy (Neurohr *et al.*, 2019), is a more important factor than decreased nutrient import rates or a suboptimal surface area-to-volume ratio, even at very large cell sizes (Mu *et al.*, 2020). However, the mechanisms underlying scaling relationships of cell size and ploidy at each step of the central dogma are only beginning to be elucidated (Swaffer *et al.*, 2021). Another underlying explanation for changes in cell fitness likely relates to how cellular metabolism scales with cell size, as several independent observations suggest that oxidative respiration decreases with size (Miettinen and Bjorklund, 2016) or ploidy (Sagi *et al.*, 2016). Investigating links among cell size, cellular physiology, and tissue physiology is likely to prove extremely fruitful as examples of connections between cell size or ploidy and organ metabolism are rife. For example, polyploidization accompanied by increases in cell size can affect the metabolic demand of organs such as the liver (Donne *et al.*, 2020) while across evolutionary timescales, the well-described scaling relationship between genome size and cell size has been hypothesized to affect overall metabolic demand of the organism (Liedtke *et al.*, 2018; Gardner *et al.*, 2020). Exploring connections between size and fitness at the cellular and organismal levels will undoubtedly yield exciting new principles of physiology, as well as how evolution has enabled life to exist at sizes that vary over 20 orders of magnitude.

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