© 2022. Published by The Company of Biologists Ltd | Journal of Cell Science (2022) 135, jcs260634. doi:10.1242/jcs.260634

MEETING REPORT

Meeting report – Cell size and growth: from single cells to the tree of life

Dimitra Chatzitheodoridou[1,*], Marco D'Ario[2,*], Ian Jones[3,*], Liliana Piñeros[4,*], Diana Serbanescu[5,*], Frank O'Donnell[6], Clotilde Cadart[7,‡] and Matthew P. Swaffer[8,‡]

ABSTRACT

In April 2022, The Company of Biologists hosted their first post-pandemic in-person Workshop at Buxted Park Country House in the Sussex countryside. The Workshop, entitled 'Cell size and growth: from single cells to the tree of life', gathered a small group of early-career and senior researchers with expertise in cell size spanning a broad range of organisms, including bacteria, yeast, animal cells, embryos and plants, and working in fields from cell biology to ecology and evolutionary biology. The programme made ample room for fruitful discussions and provided a much-needed opportunity to discuss the most recent findings relating to the regulation of cell size and growth, identify the emerging challenges for the field, and build a community after the pandemic.

[1]Institute of Functional Epigenetics, Helmholtz Zentrum München, 85764 Neuherberg, Germany. [2]Department of Cell and Developmental Biology, John Innes Centre, Norwich, NR4 7UH, UK. [3]Department of Cancer Biology, Chester Beatty Laboratories, Institute of Cancer Research, London, SW3 6JB, UK. [4]Department of Cellular and Molecular Medicine, KU Leuven, Campus Gasthuisberg O&N1bis gebouw 402-20, Herestraat 49, B-3000 Leuven, Belgium. [5]Department of Physics and Astronomy, Institute for the Physics of Living Systems, University College London, London, WC1E 6BT, UK. [6]The Company of Biologists, 94 Station Road, Histon, Cambridge, CB24 9LF, UK. [7]Molecular and Cell Biology Department, University of California, Berkeley, Berkeley, CA 94720-3200, USA. [8]Department of Biology, Stanford University, Stanford, CA 94305, USA.
*These authors contributed equally to this work

‡Authors for correspondence (clotilde.cadart@berkeley.edu; swaffer@stanford.edu)

C.C., 0000-0001-7143-2706

Introduction

Cell size is profoundly important for cellular physiology – it is specific to a given cell type and is fundamentally related to the function of the cell. For example, neurons can reach meter-long lengths to connect the central nervous system to peripheral tissues, whereas red blood cells must maintain a small size to circulate through the blood vessels.

Participants of the Workshop at Buxted Park Country House

Journal of Cell Science (2022) 135, jcs260634. doi:10.1242/jcs.260634

How cells control their size and how cell size impacts cellular physiology are century-old, yet unresolved, questions in cell biology. Central to understanding the role of cell size are the many scaling relationships that exist with size, including the scaling of size with ploidy, organelle volumes, biosynthetic rates and metabolism. First described several decades ago, researchers are only now making progress on identifying the mechanisms that underlie these long-standing problems. Importantly, several of these relationships are not just observed within a species, but also between species across the tree of life, yet the current thinking in cell biology rarely accounts for these evolutionary considerations. Thus, research from ecologists, evolutionary biologists and developmental biologists brings crucial context to these questions.

The Workshop was a faithful illustration of the unique aspects of cell size-related questions: they are relevant to all living organisms, are crucial for understanding the physiology of cells and are deeply connected to fundamental evolutionary problems. Here, we have summarized some of the key ideas and findings from the Workshop, which served as an exciting reminder that the emerging field of cell size appears in rude health and is faced with an exciting future.

Cell size homeostasis – growth and the cell cycle under the spotlight

One of the most satisfying aspects of the meeting was to see how recent work in plants, fungi and metazoans is starting to uncover the mechanisms by which cells are able to control their size. Since the 1970s it has been known that proliferating cells that are born too small to prolong their cell cycle, allowing them to grow more before dividing and thereby correct for their initially smaller size. Martin Howard (John Innes Centre, Norwich, UK) discussed how, using skinny or fat-shaped mutants, they uncovered that fission yeast always divides when cells are at the same surface area. This led them, in collaboration with Fred Chang, to identify the cell cycle activator Cdr2 as a key size-sensor, as its local concentration on the membrane increases in proportion to cell surface area (Facchetti et al., 2018). When deleting or mutating Cdr2, surface area sensing is broken but, intriguingly, cells do not lose all their size control and instead defer to a backup volume-based mechanism. Although size control is conserved across different model organisms, it appears that the underlying size-sensing molecules are not. Martin went on to discuss their collaboration with the Sablowski lab led by Marco D'Ario, showing that in plants, the G1/S inhibitor KRP4 is diluted as cells grow in G1 (D'Ario et al., 2021). Through KRP4 dilution, cell cycle progression is promoted specifically in larger cells. Ingeniously, the genome is used as a fixed standard against which to 'count' the right number of KRP4 molecules, because non-chromatin-bound KRP4 is rapidly degraded away by FBL17. In fact, the general principle of 'inhibitor dilution' was first identified by Jan Skotheim (Stanford University, USA), who started his talk by re-capping his lab's work on Whi5 dilution in budding yeast (Schmoller et al., 2015). Big or small, all cells are born with the same number of Whi5 molecules. This results in dilution of the Whi5 protein as cell size increases to promote cell division specifically in bigger cells. Jan went on to show how the same principle is at play in cultured mammalian cells, where the Retinoblastoma protein (Rb) takes the place of Whi5 (Zatulovskiy et al., 2020). In the case of Whi5, both transcription and a chromatin-based partitioning mechanism ensure cells always inherit a fixed number of Whi5 molecules regardless of their size (Swaffer et al., 2021).

To explore whether other proteins behave similarly to Whi5 or Rb, the Skotheim group have performed a proteomics analysis of size-sorted cells and found hundreds of proteins that change their concentrations with cell size (Lanz et al., 2022). One of the most striking trends in these data was that larger cells show increased expression of cellular senescence markers, foreshadowing work from Gabriel Neurohr and Jette Lengefeld on how cellular enlargement can reduce proliferation rates (discussed below). Using an impressive arrange of proteomics, phospho-proteomics and transcriptomics, Ian Jones (Institute of Cancer Research, London, UK) has mapped out how the biochemical composition of different melanoma cell lines varies with their size (Jones et al., 2022 preprint). Although changes in growth and inflammatory pathways mirrored much of what Jan presented on senescence, Ian also shared the surprising result that these changes did not materially alter the growth rate in these differently sized cancer lines. Dimitra Chatzitheodoridou (Helmholtz Centre Munich, Germany) showed that histones are another group of proteins that are produced independently of cell size, ensuring a constant protein-to-DNA stoichiometry (Claude et al., 2021). Dimitra is now investigating how histone production is linked to DNA content across different nutritional environments in budding yeast and has identified post-transcriptional regulation as a critical step for nutrient-dependent histone homeostasis.

Together, these different talks highlighted how size-dependent changes in protein concentrations are far more common in growing cells than was previously assumed. But what about in the early embryo? In amphibians, fish and invertebrates, such as the fruit fly, fertilized eggs undergo multiple rapid cycles of cell divisions with no intervening growth, which increases the DNA-to-cytoplasm ratio and triggers the mid-blastula transition (MBT), which coincides with embryonic cell cycle elongation. By measuring the DNA-bound fraction of histones, Amanda Amodeo's team (Dartmouth College, Hanover, USA) showed that in flies, a relatively fixed number of pre-loaded histone H3 is titrated against the increasing amount of nuclear DNA during these early cycles so that the free H3 concentration declines during development. Surprisingly, the Amodeo lab found that the N-terminal tail of H3 is responsible for cell cycle elongation at the MBT, because it acts as a competitive inhibitor of the Chk1 kinase independently of chromatin incorporation in the cytoplasm. Thus, as more H3 is titrated against the genome with every cycle, the total nuclear free H3 pool eventually drops below a critical threshold and Chk1 is activated, resulting in the cell cycle taking longer (Shindo and Amodeo, 2021). Inspired to examine the same problem, but in frogs, Liliana Piñeros (University of Leuven, Belgium) built droplets of *Xenopus* egg extracts of varying sizes to reconstitute cell cycle transitions *in vitro* and understand how they are influenced by cell and nuclear size. Liliana showed that cell division in larger droplets is coordinated by mitotic waves originating from individual nuclei, which become pacemakers by oscillating faster than their surroundings – the period of these oscillation is influenced by nuclear import rates.

Bruce Edgar (University of Utah, Salt Lake City, USA) introduced another central question of cell size homeostasis – how does cell cycle progression adapt to changes in growth conditions? Studying fruit fly wing progenitors and intestinal stem cells, Bruce revealed the crucial role of the translational regulation of the E2F1 transcription factor (Øvrebø et al., 2022). Upstream open reading frames in the E2F1 transcript attenuate translation, causing a slowdown in proliferation rates under poor growth conditions. However, when TOR signalling is active, this repression is alleviated to promote increased proliferation specifically when growth signalling is strong. In another example of this coupling between growth and the cell cycle, the Edgar lab characterized the

Journal of Cell Science (2022) 135, jcs260634. doi:10.1242/jcs.260634

serum-dependent effects on translation rates by ribosome profiling in RPE1 cells. This revealed that serum induces amino-acyl-tRNAs for rare codons overrepresented in DNA replication factor transcripts, supporting their model that serum stimulation promotes cell proliferation via the enhanced translation of key S phase factors. Further emphasizing the complexity of size control in multicellular organisms, Alison Lloyd (University College London, UK) recalled that in many adult animal tissues, the vast majority of cells do not proliferate and yet cell size is still very precisely regulated. Alison's lab has pioneered the use of Schwann cells, which are responsible for the myelination of peripheral axons, to study cell growth in the tissue context. As they differentiate, Schwann cells enlarge in size and increase their global transcriptional and translational output to support the biogenic demands of myelination. Alison went on to explain how HDAC2 and HDAC3 play opposing roles in switching between this highly biosynthetically active developmental state and a more basal homeostatic state, which maintains a stable cell size in adult tissue (Rosenberg et al., 2018).

In proliferating cells, size- and growth-sensing pathways ultimately feed into cyclin–Cdks – the master cell cycle regulator. Paul Nurse (Francis Crick Institute, London, UK) started his talk by explaining how the cyclin–Cdk oscillator organises the cell cycle via a gradually increasing activity so that key substrates are phosphorylated at different thresholds (Swaffer et al., 2016). Providing further support for this model, his lab has now successfully engineered yeast capable of driving mitosis with cyclin–Cdk complexes usually reserved for activating DNA replication (Basu et al., 2022). To identify new regulators that might feed size and ploidy information into the cell cycle control, Paul's lab have systematically screened for haplo-insufficient size regulators that alter size when one of the two copies in a diploid is deleted, and have also quantified the expression patterns of all the known cell cycle factors (Moris et al., 2016; Curran et al., 2022). Surprisingly, very few regulators of the G2/M transition changed their concentration with cell volume – the two exceptions being Cdc13 and Cdc25, hinting at a possible mechanism for the volume-based control that Martin Howard and Fred Chang uncovered when Cdr2 is removed.

The eukaryotic cell cycle is completely distinct from that of bacteria but in both domains of life, the cell cycle is carefully coordinated with cell size. Combining high-throughput time-lapse imaging with CRISPR screening (Lawson and Elf, 2021), Johan Elf (Uppsala University, Sweden) showed that the timing of DNA replication is tightly coordinated with cell size but not with cell division in *Escherichia coli*. Key to this phenomenon is the fact that the replication initiator factor DnaA toggles between an ATP- and ADP-bound state – the ADP-to-ATP conversion is regulated by factors in proportion to cell volume, whereas the reverse reaction is templated on DNA. This suggests an elegant model where the relative ratio of DnaA-ATP to DnaA-ADP reflects the volume-to-DNA ratio and thereby promotes genome replication whenever a cell has grown sufficiently to shift the DnaA-ATP-to-Dna-ADP ratio above a critical threshold. Bianca Sclavi (Sorbonne University, Paris, France) then presented the surprising finding that replication controls the specific growth rate in *E. coli*, because growth is not monotonic during the cell cycle but increases as DNA replicates. To better understand this pattern, Bianca analysed the activity of several promoter reporters which depend on DnaA across various growth conditions. The volume-specific activity of these promoters oscillates through time, which led Bianca to the hypothesis that DnaA acts as an oscillator coupled to cell size in single cells. How are these oscillations set? Although this is still under investigation, the interaction between DnaA and the SeqA repressor protein appears to be central, because when the binding sites of SeqA are deleted, oscillations of the promoter reporters are disrupted.

Intracellular scaling – cell size at the nexus of cellular functionality and fitness

As cells grow and change in size, so must many of their internal structures and organelles. Fred Chang (UC San Francisco, USA) recalled a classic study from Paul Nurse's lab, which demonstrated how nuclear size scales with cell size in fission yeast, regardless of DNA content (Neumann and Nurse, 2007). Following up on this observation, Fred's group have developed an elegant biophysical model where nuclear growth is set by the effects of colloid osmotic pressure. Using osmotic shock experiments and nuclear transport perturbations, they demonstrated experimentally that the nucleus behaves as a perfect osmometer, as its volume is principally set by the balance of osmotically active macromolecules between the nucleus and cytoplasm (Lemière et al., 2022). Satisfyingly, this model also quantitatively predicts the rate at which nuclear size is corrected in mutants where cells are born with an aberrant nuclear-to-cytoplasmic ratio. Rebecca Heald (UC Berkeley, USA) brought an important counterpoint to biophysical models of scaling by giving a glimpse of the diversity of intracellular scaling molecular mechanisms acquired throughout evolution in frog embryos. Combining experiments in *Xenopus* embryos and egg extracts encapsulated in droplets, Rebecca's lab showed that spindle and nuclear size are set by the cell surface area-to-volume ratio (Brownlee and Heald, 2019). Turning to the meiotic spindle, Rebecca then showed that its size regulation relies on distinct mechanisms in different frog species – whereas *X. tropicalis* and *X. laevis* species relied on the microtubule-severing protein katanin and TPX2, the distantly related pipid frog *Hymenochirus boetgerri* meiotic spindles relied on Kif2a-mediated microtubule destabilization (Miller et al., 2019).

As the mechanism for cellular and subcellular size control were being uncovered, a deceptively simple question was raised – why do cells control their size? Jette Lengefeld (University of Helsinki, Finland) investigated the consequences of cell enlargement *in vivo* using the tiny hematopoietic stem cells (HSCs). By isolating differently sized HSCs and then transplanting them into irradiated recipient mice, Jette showed that larger HSCs lose their regeneration potential due to a decreased proliferation rate that is also associated with a depletion of mitochondria and metabolites such as ATP (Lengefeld et al., 2021). Cell size is the key causal factor because when the size of large stem cells is decreased, they regain their capacity to regenerate the recipient mouse's blood system. Jette's work paved the way to a whole new series of fundamental questions focusing on the mechanisms by which cell size affects cellular physiology. The surface area-to-volume ratio is often hypothesized to be an important regulatory variable for cell growth, given that it could constrain the rate of nutrient uptake. However, when Teemu Miettinen (MIT, Cambridge, USA) induced polyploidization in mouse lymphocytes to generate cells spanning a hundred-fold range of sizes, he found that their mass-normalized growth rate was constant (Mu et al., 2020). To explain this surprising result, Teemu then investigated the size-dependency of cell surface protein content using new measurement techniques. This revealed that cell surface proteins increase at the same rate as cell volume, suggesting an increased membrane folding in larger cells that keeps surface-to-volume ratio independent of size. Crucially, in these experiments when cell size increases, so does DNA content, meaning the cell

Journal of Cell Science (2022) 135, jcs260634. doi:10.1242/jcs.260634

volume-to-DNA ratio is roughly constant. In contrast, Gabriel Neurohr (ETH Zurich, Switzerland) studied budding yeast cells arrested in G1 and the DNA content of these cells remained fixed while cell volume increased massively. In this context, cellular growth and biosynthesis break down, cytoplasmic RNA and protein are diluted, and cells become prone to senescence, thus emphasizing that the size-to-DNA ratio, not cell size per se, is key for efficient growth (Neurohr et al., 2019). Gabriel also showed that doubling the DNA content of yeast cells, either via polyploidy or just by adding large amounts of junk DNA, has a similar effect on increasing cell size. This intriguing result suggests that, at least in budding yeast, the size-to-DNA ratio is kept roughly constant across ploidies because cell size is directly coupled to the DNA amount regardless of whether it is coding or not. Despite the deleterious effects of the dramatic increases in size highlighted by Gabriel, cells are remarkably successful at scaling and maintaining processes in their physiological size range. Matthew Swaffer (Stanford University, USA) investigated the mechanisms underlying the long-reported linear scaling between mRNA amounts and cell volume. He identified RNA polymerase II (RNAPII) as the major limiting factor for increasing global mRNA synthesis in larger cells and explained how the global transcription rate is determined by the underlying equilibrium kinetics of the different nuclear RNAPII populations (Swaffer et al., 2022 preprint). However, this transcriptional scaling is not perfectly proportional to cell size and Matthew identified an additional compensating feedback on mRNA turnover that stabilizes transcripts in larger cells. How does this fit in with conclusions from Gabriel and Teemu that the size-to-DNA ratio is key for cell growth? Well, DNA content is also a key parameter in this model, and Matthew showed that transcription occurs at a higher overall rate in diploid cells compared to similarly sized haploids, which might explain why increased ploidy is able to sustain efficient growth at larger cell sizes.

Polyploidy is also key in *Arabidopsis* sepals, where a fraction of cells become giant to promote tissue curvature. Adrienne Roeder's group (Cornell University, Ithaka, USA) showed that in sepals, endoreduplication is triggered when the concentration of the ATML1 transcription factor, which varies stochastically among cells, reaches a critical threshold (Meyer et al., 2017). Adrienne's team have gone on to identify very long chain fatty acids (VLCFA) as a key non-proteinaceous regulator of ATML1, which together control endoreplication via the regulation of the Cdk inhibitor LGO. Surprisingly, endoreduplication can be reversible in sepals, challenging the dogma that once cells endoreplicate they cannot revert to a lower ploidy. Marco D'Ario also addressed the contribution of cells to the mechanical properties of plants, but from a paleontology perspective. Through 3D computational reconstruction, Marco studied cell size distribution in eophyte fossils, a 400 million-year-old extinct taxon of non-vascular plants. Marco has started exploring possible functional consequences of the diverse forms he observes, such as the identification of putative pressure-driven spore release mechanisms in these ancient organisms.

Despite the many physiological situations where ploidy increases, whole-genome duplication (WGD) is also – somewhat paradoxically – an early step in many cancers. To shed light on this, Renata Basto (Institut Curie, Paris, France) artificially induced tetraploidization by different means in human cell lines and observed a major spike in DNA damage during the first S phase that followed tetraploidization (Gemble et al., 2022). Renata showed that when the ploidy doubles, certain factors required for DNA replication do not scale up. Simply elongating G1 phase to allow cells to accumulate more S phase components was sufficient to rescue the genetic instability in tetraploid cells, as was upregulating key DNA replication factors. Thus, the absence of scaling of replication factors with ploidy in the very first S phase following WGD is a major source of DNA damage and could help explain the link between WGD and tumorigenesis. Zuzana Storchová (University of Kaiserlautern, Germany) expanded on the theme of protein expression scaling with cell ploidy by analysing yeast strains ranging from haploids to tetraploids (Yahya et al., 2021 preprint). Proteomic and transcriptomic analyses revealed that many proteins alter their expression with changes in ploidy, whereas the mRNAs that encode them do not. Translational machinery was one of the main groups to be downregulated with increased ploidy and this was associated with lower mTOR signalling and the upregulation of the Tup1 transcriptional regulator. Zuzana proposed that these changes in translational machinery reduce the relative protein synthesis rate in higher ploidy cells, explaining why tetraploids have a lower protein-to-DNA ratio than haploids.

Metabolism and cell size – a common thread from cellular to whole organism scaling?

Although our understanding about how cellular functions are affected by changes in cell size, ploidy and the DNA-to-cytoplasm ratio progresses, a new question emerges – how do these changes affect higher levels of organization, such as the tissue or organism scale? Clotilde Cadart (UC Berkeley, USA) developed a novel approach to study the consequences of polyploidy and cell size increase at the organism level by generating polyploid *Xenopus* embryos. In triploid *X. laevis* and *X. borealis* embryos, she observed that whole-organism metabolic rate, assessed by measuring the oxygen consumption rate, was reduced compared with that in diploids. She then presented a mathematical framework to decipher how the energetic costs of growth, proliferation and maintenance change with cell number and ploidy in embryos. To bring an evolutionary perspective to her findings, Clotilde is also comparing the onset of scaling relationships between genome size, cell size and developmental rate in *Xenopus laevis* and *Xenopus longipes* species, which evolved following ancestral polyploidization events (and have 4N and 12N genomes, respectively) (Miller et al., 2022). How could metabolism change with size? Together with Jette's work, Clotilde's results highlight the importance of this question (Cadart and Heald, 2022).

Using *E. coli*, Christine Jacobs-Wagner (Stanford University, USA) made a compelling case for the importance of studying ATP regulation at the single-cell level. Combining a ratiometric ATP sensor with a high throughput single-cell tracking protocol, her lab identified high cell-to-cell variability in ATP concentrations, a phenomenon that was most pronounced in nutrient-rich conditions (Lin and Jacobs-Wagner, 2022). Using mutants in the acetate fermentation pathway and media switches, Christine argued that temporal dynamics of overflow metabolism contributes to these fluctuations. Remarkably, the amplitude of the fluctuations, but not the average ATP concentration, is negatively correlated with single-cell growth rates, suggesting that cells with more stable ATP levels are those most capable of optimal growth. To further understand the energy budget of bacteria, Diana Serbanescu (University College London, UK) has developed theoretical models accounting for nutrient import, growth, division, metabolism, shape maintenance and energy loss (Serbanescu et al., 2021). Diana's approach is based on optimization of the physiological energy assimilation to ultimately maximize cellular fitness for proliferation. Her work uncovers feedback motifs for bacteria's adaptive response to chemical and mechanical perturbations.

Understanding whether and how genome size and cell size are linked to organismal variables across species would shed light on the type of constraints on genome size evolution. Ivan Gomez-Mestre (Doñana Biological Station, Seville, Spain), an ecologist and evolutionary biologist, brought a crucial perspective to this question. Comparing hundreds of amphibian species, he showed that the duration of the developmental period is positively associated with genome size across frogs and toads (Liedtke et al., 2018). Since genome size and cell size are correlated across species, it is tempting to speculate that cell size-dependent changes in physiology might mediate this correlation. Ivan's talk highlighted the breadth of questions that can be investigated in amphibians, such as the interactions among environmental conditions, evolutionarily acquired plasticity to environmental variability, gene expression or embryo development; this sparked the curiosity of many cell biologists in the audience who are hoping to bridge the gap between cell and organismal physiology. Douglas Glazier (Juniata College, Huntingdon, USA) recalled the varied and complex relationships between cell size and different organismal traits. Focusing on how cell size and number relate to metabolic rates, growth, development and propagule production, he proposed that we consider cell size as a 'hub trait' for organism physiology (Glazier, 2022). Douglas went on to speculate about how changes in cell size might be an integral part of biological responses to changing environments, including both phenotypically plastic multi-trait responses and the genotypic evolution of integrated adaptive traits at the population level.

Going beyond cross-species analyses and finding an experimental angle to understand the relationship between size, growth and metabolism at the organismal scale is no small challenge, but several attendees presented new and exciting experimental approaches. To test whether and how organ growth is constrained by energy consumption during *Drosophila* development, Youmna Atieh (IBDM, Marseille, France) proposed a novel experimental strategy where she will dissect and perform single-organ microcalorimetry measurements. In flatworms, which can grow or 'de-grow' depending on food availability even at the adult stage, Jochen Rink (Max Plank Institute for Multidisciplinary Sciences, Goettingen, Germany) aims to elucidate how body size, growth rate and organism physiology are linked. Jochen showed that body size is a very strong predictor of overall gene expression, independently of the life history of the animal and whether it reached its size by growing, shrinking or regeneration. He then identified a conserved signalling molecule that itself is body size dependent and explains a major subset of size-dependent gene expression patterns. Thus, this signalling pathway appears to operate as a systemic regulator that controls the rates of both cell proliferation and organism growth to couple organism size to its physiological state. Using *Caenorhabditis elegans*, Benjamin Towbin (University of Bern, Switzerland) asked whether and how size homeostasis is achieved at the organism level during development. He showed that in developing larvae, heterogeneities in body sizes are compensated for by BLMP-1, an oscillatory transcription factor that negatively couples developmental time to growth rate (Stojanovski et al., 2022b). Next, turning to organ size homeostasis, Benjamin observed that pharynx size control is robust, showing 'adder'-like properties (Stojanovski et al., 2022a preprint). To ask whether such control is tissue-autonomous or systemic, his lab performed tissue-specific inhibition of the mTOR activator RagA in the pharynx and hypodermis. This caused a strong systemic response, thus suggesting that mechanisms must exist to regulate growth across tissues and ensure robust pharynx-to-body size proportions.

Altogether, talks from Benjamin, Jochen, Youmna, and Clotilde point to a new frontier for cell size biologists – understanding how organismal physiology is set and results from its constituent organs and cells. In this endeavour, the theory of scaling studied by Douglas Glazier or Van Savage (Savage et al., 2007; Brummer and Savage, 2021) – who was unfortunately unable to join the meeting – brings important conceptual tools whereas evolutionary approaches and experiments in amphibians presented by Ivan Gomez-Mestre provide a crucial context for such an ambitious undertaking.

Concluding remarks

On the last evening, we gathered for a conversation where each attendee presented the questions that had most sparked their interest. The breadth of the questions discussed was a testimony of the diversity of fundamental processes that cell size touches upon. At the cellular scale, the precise molecular mechanisms allowing the coordination of cell cycle progression and growth to maintain size homeostasis are being elucidated and it was suggested that future research might focus on how conserved size control patterns are throughout evolution. At the subcellular scale, the regulation of organelles and processes such as biosynthesis or metabolism, as a function of cell size, appears as the new hot topic, opening many unanswered questions on the relationship between cell size and cell physiology. Directly related to this, a new frontier emerges – the connection between cell physiology and tissue or organismal physiology, with novel experimental approaches currently being developed in amphibians, planarians, worms and flies. The conversation also insisted on the need to develop multidisciplinary approaches to support cell biologists. For the size scaling relationships that are conserved across multiple taxa, biophysical models will likely be key to uncovering the underlying principles. Evolutionary biology and phylogeny approaches bring crucial context to our questions. Finally, developing connections with experts in cellular and organismal metabolism might become crucial to unlock some of the current open questions. Overall, the cell size community is still young but is characterized by a pioneering, creative and collaborative spirit, which places it in a unique place to bridge the gap across diverse fields of biology.

Acknowledgements

We would like to thank the Company of Biologists for hosting and sponsoring this workshop as well as everyone at the Company of Biologists for their hard work organising the workshop and making it such a success. We would also like to thank all the attendees for bringing such a cordial and collegiate atmosphere to the meeting and all the staff at Buxted House for their warm welcome and for ensuring everything ran so smoothly.

Competing interests

The authors declare no competing or financial interests.

References

Basu, S., Greenwood, J., Jones, A. W. and Nurse, P. (2022). Core control principles of the eukaryotic cell cycle. *Nature* **607**, 381-386. doi:10.1038/s41586-022-04798-8

Brownlee, C. and Heald, R. (2019). Importin α partitioning to the plasma membrane regulates intracellular scaling. *Cell* **176**, 805-815.e8. doi:10.1016/j.cell.2018.12.001

Brummer, A. B. and Savage, V. M. (2021). Cancer as a model system for testing metabolic scaling theory. *Front. Ecol. Evol.* **9**, 691830. doi:10.3389/fevo.2021.691830

Cadart, C. and Heald, R. (2022). Scaling of biosynthesis and metabolism with cell size. *Mol. Biol. Cell* **33**, 1-6. doi:10.1091/mbc.E21-12-0627

Claude, K.-L., Bureik, D., Chatzitheodoridou, D., Adarska, P., Singh, A. and Schmoller, K. M. (2021). Transcription coordinates histone amounts and genome content. *Nat. Commun.* **12**, 4202. doi:10.1038/s41467-021-24451-8

Curran, S., Dey, G., Rees, P. and Nurse, P. (2022). A quantitative and spatial analysis of cell cycle regulators during the fission yeast cycle. *Proc. Natl Acad. Sci. USA* **119**, e2206172119. doi:10.1073/pnas.2206172119

D'Ario, M., Tavares, R., Schiessl, K., Desvoyes, B., Gutierrez, C., Howard, M. and Sablowski, R. (2021). Cell size controlled in plants using DNA content as an internal scale. *Science* **372**, 1176-1181. doi:10.1126/science.abb4348

Facchetti, G., Knapp, B., Flor-Parra, I., Chang, F. and Howard, M. (2018). Reprogramming Cdr2-dependent geometry-based cell size control in fission yeast. *Curr. Biol.* **29**, 350-358.e4. doi:10.1016/j.cub.2018.12.017

Gemble, S., Wardenaar, R., Keuper, K., Srivastava, N., Nano, M., Macé, A.-S., Tijhuis, A. E., Bernhard, S. V., Spierings, D. C. J., Simon, A. et al. (2022). Genetic instability from a single S phase after whole-genome duplication. *Nature* **604**, 146-151. doi:10.1038/s41586-022-04578-4

Glazier, D. S. (2022). How metabolic rate relates to cell size. *Biology* **11**, 1106. doi:10.3390/biology11081106

Jones, I., Dent, L., Higo, T., Roumeliotis, T., Garcia, M. A., Shree, H., Choudhary, J., Pedersen, M. and Bakal, , C. (2022). Characterization of proteome-size scaling by integrative omics reveals mechanisms of proliferation control in cancer. *bioRxiv*, 2022.06.21.496989.

Lanz, M. C., Zatulovskiy, E., Swaffer, M. P., Zhang, L., Ilerten, I., Zhang, S., You, D. S., Marinov, G., Mcalpine, P., Elias, J. E. et al. (2022). Increasing cell size remodels the proteome and promotes senescence. *Mol. Cell* **82**, 3255-3269.e8. doi:10.1016/j.molcel.2022.07.017

Lawson, M. and Elf, J. (2021). Imaging-based screens of pool-synthesized cell libraries. *Nat. Methods* **18**, 358-365. doi:10.1038/s41592-020-01053-8

Lemière, J., Real-Calderon, P., Holt, L. J., Fai, T. G. and Chang, F. (2022). Control of nuclear size by osmotic forces in Schizosaccharomyces pombe. *eLife* **11**, e76075. doi:10.7554/eLife.76075

Lengefeld, J., Cheng, C.-W., Maretich, P., Blair, M., Hagen, H., Mcreynolds, M. R., Sullivan, E., Majors, K., Roberts, C., Kang, J. H. et al. (2021). Cell size is a determinant of stem cell potential during aging. *Sci. Adv.* **7**, eabk0271. doi:10.1126/sciadv.abk0271

Liedtke, H. C., Gower, D. J., Wilkinson, M. and Gomez-Mestre, I. (2018). Macroevolutionary shift in the size of amphibian genomes and the role of life history and climate. *Nat. Ecol. Evol.* **2**, 1792-1799. doi:10.1038/s41559-018-0674-4

Lin, W. H. and Jacobs-Wagner, C. (2022). Connecting single-cell ATP dynamics to overflow metabolism, cell growth, and the cell cycle in Escherichia coli. *Curr. Biol.* **32**, 3911-3924.e4. doi:10.1016/j.cub.2022.07.035

Meyer, H. M., Teles, J., Formosa-Jordan, P., Refahi, Y., San-Bento, R., Ingram, G., Jönsson, H., Locke, J. C. W. and Roeder, A. H. K. (2017). Fluctuations of the transcription factor atml1 generate the pattern of giant cells in the arabidopsis sepal. *eLife* **6**, e19131. doi:10.7554/eLife.19131

Miller, K., Cadart, C. and Heald, R. (2022). Dodecaploid Xenopus longipes provides insight into the emergence of size scaling relationships during development. *bioRxiv*, 2022.08.16.504201. doi:10.1101/2022.08.16.504201

Miller, K. E., Session, A. M. and Heald, R. (2019). Kif2a scales meiotic spindle size in Hymenochirus boettgeri. *Curr. Biol.* **29**, 3720-3727.e5. doi:10.1016/j.cub.2019.08.073

Moris, N., shrivastava, J., Jeffery, L., Li, J.-J., Hayles, J. and Nurse, P. (2016). A genome–wide screen to identify genes controlling the rate of entry into mitosis in fission yeast. *Cell Cycle* **15**, 3121-3130. doi:10.1080/15384101.2016.1242535

Mu, L., Kang, J. H., Olcum, S., Payer, K. R., Calistri, N. L., Kimmerling, R. J., Manalis, S. R. and Miettinen, T. P. (2020). Mass measurements during lymphocytic leukemia cell polyploidization decouple cell cycle- and cell size-dependent growth. *Proc. Natl Acad. Sci. USA* **117**, 15659-15665. doi:10.1073/pnas.1922197117

Neumann, F. R. and Nurse, P. (2007). Nuclear size control in fission yeast. *J. Cell Biol.* **179**, 593-600. doi:10.1083/jcb.200708054

Neurohr, G. E., Terry, R. L., Lengefeld, J., Bonney, M., Brittingham, G. P., Moretto, F., Miettinen, T. P., Vaites, L. P., Soares, L. M., Paulo, J. A. et al. (2019). Excessive cell growth causes cytoplasm dilution and contributes to senescence. *Cell* **176**, 1083-1097.e18. doi:10.1016/j.cell.2019.01.018

Øvrebø, J. I., Bradley-Gill, M.-R., Zielke, N., Kim, M., Marchetti, M., Bohlen, J., Lewis, M., Van Straaten, M., Moon, N.-S. and Edgar, B. A. (2022). Translational control of E2f1 regulates the Drosophila cell cycle. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2113704119. doi:10.1073/pnas.2113704119

Rosenberg, L. H., Cattin, A.-L., Fontana, X., Harford-Wright, E., Burden, J. J., White, I. J., Smith, J. G., Napoli, I., Quereda, V., Policarpi, C. et al. (2018). HDAC3 regulates the transition to the homeostatic myelinating schwann cell state. *Cell Reports* **25**, 2755-2765.e5. doi:10.1016/j.celrep.2018.11.045

Savage, V. M., Allen, A. P., Brown, J. H., Gillooly, J. F., Herman, A. B., Woodruff, W. H. and West, G. B. (2007). Scaling of number, size, and metabolic rate of cells with body size in mammals. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 4718-4723. doi:10.1073/pnas.0611235104

Schmoller, K., Turner, J. J., Kõivomägi, M. and Skotheim, J. M. (2015). Dilution of the cell cycle inhibitor Whi5 controls budding yeast cell size. *Nature* **526**, 268-272. doi:10.1038/nature14908

Serbanescu, D., Ojkic, N. and Banerjee, S. (2021). Cellular resource allocation strategies for cell size and shape control in bacteria. *FEBS J.* doi:10.1111/febs.16234

Shindo, Y. and Amodeo, A. A. (2021). Excess histone H3 is a competitive Chk1 inhibitor that controls cell-cycle remodeling in the early Drosophila embryo. *Curr. Biol.* **31**, 2633-2642.e6. doi:10.1016/j.cub.2021.03.035

Stojanovski, K., Gheorghe, I., Lanjuin, A., Mair, W. B. and Towbin, B. D. (2022a). Ultra-sensitive coupling between organ growth and size by YAP-1 ensures uniform body plan proportions in C. elegans. *bioRxiv*, 2022.09.05.506666. doi:10.1101/2022.09.05.506666

Stojanovski, K., Großhans, H. and Towbin, B. D. (2022b). Coupling of growth rate and developmental tempo reduces body size heterogeneity in C. elegans. *Nat. Commun.* **13**, 1-14. doi:10.1038/s41467-022-29720-8

Swaffer, M. P., Jones, A. W., Flynn, H. R., Snijders, A. P. and Nurse, P. (2016). CDK substrate phosphorylation and ordering the cell cycle. *Cell* **167**, 1750-1761.e16. doi:10.1016/j.cell.2016.11.034

Swaffer, M. P., Kim, J., Chandler-Brown, D., Langhinrichs, M., Marinov, G. K., Greenleaf, W. J., Kundaje, A., Schmoller, K. M. and Skotheim, J. M. (2021). Transcriptional and chromatin-based partitioning mechanisms uncouple protein scaling from cell size. *Mol. Cell* **81**, 4861-4875.e7. doi:10.1016/j.molcel.2021.10.007

Swaffer, M. P., Marinov, G. K., Zheng, H., Tsui, C. Y., Jones, A. W., Greenwood, J., Kundaje, A., Greenleaf, W. J., Reyes-Lamothe, R. and Skotheim, J. M. (2022). RNA polymerase II dynamics and mRNA stability feedback determine mRNA scaling with cell size. *bioRxiv*, 2021.09.20.461005. doi:10.1101/2021.09.20.461005

Yahya, G., Menges, P., Ngandiri, D. A., Schulz, D., Wallek, A., Kulak, N., Mann, M., Cramer, P., Savage, V., Raeschle, M. et al. (2021). Scaling of cellular proteome with ploidy. *bioRxiv*, 2021.05.06.442919. doi:10.1101/2021.05.06.442919

Zatulovskiy, E., Zhang, S., Berenson, D. F., Topacio, B. R. and Skotheim, J. M. (2020). Cell growth dilutes the cell cycle inhibitor Rb to trigger cell division. *Science* **369**, 466-471. doi:10.1126/science.aaz6213