

## Voices

## More Metabolism!

To celebrate our Focus Issue, we asked a selection of researchers working on different aspects of metabolism what they are excited about and what is still to come. They discuss emerging concepts, unanswered questions, things to consider, and technologies that are enabling new discoveries, as well as developing and integrating approaches to drive the field forward.



**James A. Olzmann**  
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**Complexity, compartments, and crosstalk**

Metabolism is often represented as an intricate network of crisscrossing metabolic pathways reminiscent of a San Francisco Bay Area street map, complete with metabolite highways, busy intersections, and sometimes, traffic jams. While this imagery captures a portion of the complexity of metabolism, current paradigms move beyond these 2-dimensional maps to emphasize the spatial organization of metabolic pathways within cells. In the crowded intracellular environment, membrane-bound and membraneless organelles are necessary to compartmentalize metabolic processes. Moreover, these organelles house factors that permit the regulated transfer of metabolites at organelle contact sites, and they also contain large dynamic protein assemblies that facilitate efficient metabolite channeling. In recent years, research in this field has yielded numerous exciting discoveries related to the cell biology of metabolism, such as new organelle tethering complexes, lipid transfer proteins, and mechanisms of organelle and membrane homeostasis. We have likely only scratched the surface when it comes to understanding the spatial organization and regulation of metabolism, and there is still much to learn about how cells sense and adapt to metabolic fluctuations as well as how these processes differ among cell types and under disease states.

There are several technological advances that continue to be vital for dissecting the most pressing questions in cellular metabolism. Advances in microscopy, including super-resolution, expansion, electron, and nonlinear optical microscopy, enable the observation of organelle architecture and metabolism in unprecedented detail. Additionally, chemical biology approaches such as proximity labeling, genetically encoded tools to manipulate metabolism, fluorescence-based metabolic sensors, and molecular glues, allow us to visualize and manipulate metabolic reactions. However, there remains a tremendous need to develop new imaging approaches for the direct tracking of metabolites and new chemical biology tools to fully reflect the vast scope of cellular metabolites. Furthermore, there is incredible potential in the integration of systems-level methodologies with cell biology and biochemistry approaches, such as the combination of genome-wide CRISPR-Cas9 screens with reporter systems (e.g., fluorescence-based metabolic sensors) to discover new factors and facets of metabolism.

Technological and biological advances go hand-in-hand, enabling progress and pushing the frontiers of discovery. Applying emerging technologies and integrating systems level “omics” data to achieve a comprehensive and mechanistic understanding of metabolic organization across all scales, from subcellular to organismal, is bound to uncover new principles of biology and enable more effective therapeutic targeting of metabolism.



**Sarah-Maria Fendt**  
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### Connecting input with phenotype

Metabolism—the entirety of biochemical reactions that occur in a living cell—is often considered a dry, complex, and all-known subject. However, when I think about metabolism, I see the engine that keeps all processes in a cell running, I see the network that connects information and input to phenotype, and I see the mystery of a fine-tuned and precise communication arising from a seemingly chaotic mix of small molecules, ions, and proteins (and other macromolecules). Although knowing that more than one evolutionary theory exists, it seems likely to me that metabolism is at the origin of life.

Based on my own research on metabolism, I find it most amazing under which circumstances cellular metabolism is still functioning normally—just thinking about the obvious differences in nutrient exposure, energy demand, and environment living organisms are facing. In this respect, it is striking how little we know about the underlying mechanisms and causalities that allow metabolic homeostasis or destroy it. Once homeostasis is compromised, aberrant metabolism causes disease or aids disease progression. This shift in metabolism associated with disease and progression is what my laboratory studies with a focus on cancer. Our vision is to mechanistically understand and modulate the intertwined functionality of metabolism in cancer cells to eradicate them and to reestablish homeostasis. Our hope is that by targeting metabolism we prevent or inhibit disease progression toward metastasis formation.

When working on a devastating disease such as cancer, impact and application are obvious and a balance between translational and fundamental research a necessity. I therefore strongly believe that a mechanistic and functional understanding of metabolism will bring us closer to aiding patients by providing new therapeutic strategies to manage, cure, and prevent diseases such as cancer.



**Yatrik Shah**  
University of Michigan, Ann Arbor, USA

### Microbes and micronutrients

What excites me about metabolism research is shaped by my lab's interest at the nexus of the microbiome, oxygen, and iron coordination in normal physiology and gastrointestinal diseases including cancer. Understanding heterocellular metabolite exchange that exists between the microbiota and host cells is still in its infancy. Microbially derived butyrate is produced in millimolar levels and is a major metabolite that maintains colonic epithelial energetics. The gut microbiota is the most dense and diverse consortium of bacteria that exist. Metagenomics and metabolomics studies demonstrate that the gut microbiota produce a myriad of uncharacterized metabolites. How gut microbial metabolites integrate and shape host metabolism (locally and peripherally) and dictate disease progression is unclear. Moreover, the bidirectional aspects of the metabolite exchange are also very important but even less well studied. Understanding if host metabolism can alter microbial communities via competitive or cooperative metabolic interactions will lead to better understanding of how dysbiotic communities emerge and alter disease pathogenesis.

Additionally, how is cellular metabolism coupled to micronutrient metabolism? Biologically relevant divalent metals, such as iron, are essential binding cofactors for 30% of all proteins and 40% of enzymatic cellular reactions require metals. Indeed, processes such as DNA synthesis, epigenetic regulation, mitochondrial respiration, lipid metabolism, redox balance and cell death require metals. Currently, mass spectrometry approaches can quantitate trace levels of divalent metals. Understanding how micronutrients regulate and integrate into classic cellular energetics fueled by glucose, amino acids, and lipids will identify new areas of how cellular metabolism is regulated.

Current mass spectrometry platforms can detect and quantitate thousands of metabolites and dozens of micronutrients in a cell, tissue, or biofluid. This has led to unparalleled mapping of metabolic states, plasticity, and redundancy of metabolic pathways. The analytical challenges to the points mentioned above will be facilitated by technologies analogous to the rise of genomics. These include higher throughput, higher sensitivity (single cell approaches), spatial resolution, better processing of untargeted workflows, and open-source data analysis tools.



**Karen Vousden**  
The Francis Crick Institute, United Kingdom

### Toward precision nutrition

An appreciation of the link between diet and health, with instructions on what to eat to stave off disease, has been with us since ancient times. Modern-day governments and health organizations produce a tsunami of advice to promote healthy eating, and while much of this is aimed at prevention, increasing attention is being paid to potentially therapeutic effects of diet. Cancer patients often seek diets that might limit their disease or augment therapy, but there has been little mechanistic understanding of how different diets might function to achieve these goals. This is now changing with the resurgence in research into cancer metabolism beginning to reveal the nutritional requirements of cancer cells and how these are influenced by the organ of origin, the genetics of the cancer, the tumor environment, and the therapeutic intervention. An understanding of the complex network of interdependencies and vulnerabilities that are exhibited by different cancers is paving the way for the implementation of precision nutrition, in which the multiple characteristics of a given tumor can be matched with selective limitation or supplementation of specific nutrients such as carbohydrates or selected amino acids. It's true that, so far, demonstrable effects have been largely seen in animal models, but these approaches are now being trialed in cancer patients, who are frequently highly motivated to change their diet and regain some feeling of control over their treatment. In the future, personalized diets that enhance responses to therapy may join surgery, chemo, radiation, and immunotherapy as mainstream treatment options.



**Navdeep Chandel**  
Northwestern University, USA

### Mitochondrial metabolites

"Mitochondrial dysfunction" has been proposed as a causal mechanism to explain normal aging as well as a myriad of diseases, including autoimmunity, neurodegeneration, diabetes, fibrosis, and cancer. However, there are two questions that remain unanswered. (1) What does mitochondrial dysfunction mean? and (2) Does mitochondrial dysfunction occur in normal aging and age-related diseases? For most biologists, the term mitochondrial dysfunction is synonymous with decreased ATP production, leading to loss of cellular function and tissue degeneration. However, empirical data does not support the premise that decreased mitochondrial ATP production is rate limiting for cell survival, proliferation, or function in normal aging or most common diseases, e.g., cancer or diabetes. Recent advances in metabolomics have revealed that mitochondrial metabolism is reprogrammable and that metabolite production can be altered to dictate normal cell function *in vivo*, independently of the mitochondria's ability to generate ATP. Furthermore, alterations in metabolites can cause a decline in normal cell function *in vivo*. Therefore, defining which metabolites control normal cell function and how changes in metabolite abundance affect cell physiology and pathology is an exciting new topic to explore. It is important to note that there are several diseases that are directly linked to severe impairment of the mitochondrial electron transport chain (ETC) due to mutations in essential ETC genes. These include neurological pathologies such as the pediatric disease Leigh syndrome, as well as adult Parkinson's disease, both of which are linked to impairment of mitochondrial complex I function. It is possible that these neurological disorders are due to aberrant production of mitochondrial metabolites that are pathogenic to neuronal cells. There is a precedent in the field of inborn errors of metabolism, many of which display neuronal pathologies, that symptomatic disease causally arises from alterations in metabolites. Going forward, it will be exciting to explore whether many common diseases are actually due to the accumulation of pathogenic metabolites (i.e., metabolic toxicity) or decrease in physiologic metabolites that control normal cell function.



**Tiffany Horg**  
ShanghaiTech University, Shanghai, China

### Modulation of inflammation

As highlighted in the ongoing COVID-19 pandemic, sustained inflammatory responses during infection trigger cytokine storm and tissue damage and may play a bigger role in mortality than the direct effects of the pathogen. While metabolism is known to regulate the induction of inflammatory responses, recent studies indicate that it may have an even more prominent role in their suppression. For example, in macrophages exposed to microbial stimuli, a shutdown of oxidative metabolism supports a host-protective reduction of inflammatory gene expression. In a different field, metabolism has been recently implicated in mediating one form of host defense called disease tolerance. Host defense has traditionally been viewed through the lens of disease resistance, in which host effector mechanisms like T cell-mediated cytotoxicity and inflammatory responses coordinate pathogen elimination at the cost of inducing tissue damage, while recent studies highlight the complementary role of a distinct form of host defense called disease tolerance. Disease tolerance relies on the body's ability to tolerate the tissue damage induced by pathogen virulence factors and, perhaps more importantly, by disease resistance mechanisms to survive pathogen infection. In severe cases of COVID-19, for example, the ability to tolerate cytokine storm through disease tolerance mechanisms is likely to correlate with favorable outcome. Intriguingly, many disease tolerance mechanisms that have been identified in this emerging field seem to be inextricably linked to metabolism. I expect and look forward to many more studies in the next few years to uncover the mechanisms by which metabolism enforces suppression of inflammatory response and promotes disease tolerance for overall host fitness during pathogen infection.



**Nika Danial**  
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### Factoring in fuel flexibility

Different cell states have anabolic and catabolic requirements that are fulfilled by metabolism of specific nutrients/fuel substrates. These alter cellular energy, reductive power, and biosynthetic intermediates, thereby modulating a myriad of homeostatic processes. Evidence indicates that the cells' fuel choice can influence transitions between quiescence and proliferation, produce resistance or sensitivity to oxidative stress, facilitate DNA and tissue repair, and allow metabolic adaptations to nutrient changes. Furthermore, metabolism of specific fuel substrates can affect cell identity and behavior through programmatic alterations in gene expression and epigenetic modifications. However, our understanding of metabolic mechanisms underlying these effects is currently incomplete and continues to evolve. What are the cell autonomous and non-autonomous underpinnings of cellular fuel utilization patterns? How does processing of fuel substrates translate into anabolic and catabolic signals that shape cellular and systemic physiology? How does fuel flexibility and fuel choice factor into cellular stress responses? The answer to these questions will not only advance our basic understanding of metabolic regulation but also unveil translational insights into metabolic contributions to disease pathogenesis. It would be particularly important to determine whether reprogramming of fuel utilization patterns is a cause or a consequence of alterations in cell fate, function, and stress adaptation. Within the context of fuel choice, substrate supply and substrate access are important control points that can be heavily influenced by metabolic compartmentalization/channeling, spatial organization of metabolic enzymes in macro-molecular complexes and their allosteric regulation, functional crosstalks with cellular organelles such as mitochondria and lipid droplets, as well as the cellular microenvironment. Unraveling these connections at the molecular level will provide fertile ground for new discoveries in metabolic biology and metabolic biochemistry.



**Benjamin Tu**  
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USA

### Considering the metabolic state

Advances in -omics technologies have enabled measurements of transcripts, proteins, the epigenome, and more at unprecedented scale and resolution. However, are there other variables that could affect our interpretation of these data? What might we be missing? For example, does it matter whether cells were sampled in a fed or fasted state or during a particular time of day? Genes specify which RNAs and proteins can be expressed. Many of these proteins encode enzymes that perform cellular metabolism—the very reactions and transformations that enable life. These small molecule metabolites carry information about the status of cells and their environments, which must subsequently inform expression programs and other decisions they must make. Which aspects of metabolism are relevant in this regard? Real-time and static reporters of metabolism, both direct and indirect, represent important areas of future research to enable interpretation of this additional dimension of metabolism. Furthermore, elucidating the specific mechanisms by which metabolism can feed back to regulate cellular processes will reveal cool surprises and new opportunities for therapeutic intervention. Let's not overlook the influence of the metabolic state.



**Heather Christofk**  
University of California, Los Angeles, USA

### Sensing metabolic signals

With the mounting evidence that metabolites impact cancer growth and metastasis, I am increasingly interested in how metabolic cues and nutrients instruct tissue stem cell decisions and development. Intuitively, it makes sense that tissue stem cells would use metabolites as guiding factors for decision-making: microorganisms rely upon nutrient cues from the environment to decide whether to grow, divide, or migrate. Other signaling molecules such as hormones, growth factors, and cytokines, also evolved to regulate cell fate and function. However, in addition to these signals, tissue stem cells can sense nutrients in their environment. These metabolic signals can be altered by diet, extracellular matrix remodeling, or secretion by local cells. It will be exciting for the field to further examine how tissue stem cells respond to these metabolic cues and nutrients to influence cell fate decisions. Better technologies to evaluate *in vivo* metabolism will help the field address how local changes in nutrient levels and tissue stem cell metabolic state impact tissue homeostasis and repair. Most important will be the development of technologies that enable cell type-specific *in vivo* metabolic measurements, since tissue stem cells are often rare populations within tissues. I look forward to additional mechanistic studies that will link specific metabolites to direct effects on signaling pathway enzymes, transcription factors, and epigenetic enzymes. Defining the impact metabolism has on tissue stem cell decisions, tissue homeostasis, and regeneration could help guide precision nutrition approaches to accompany pharmaceuticals for better patient outcomes.



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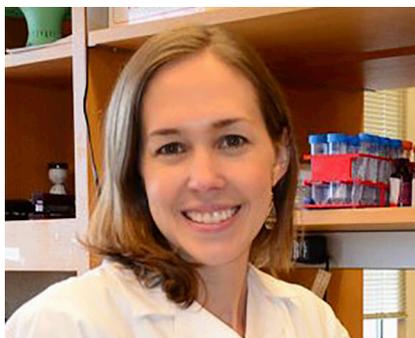
### Why so much glucose?

We have long known that cancer and other proliferating cells, as well as select non-proliferating cells, consume far more glucose than most other cells, yet our understanding of this phenotype remains mired in speculation and controversy. Glucose metabolism can contribute to many aspects of cell physiology, supporting synthesis of ATP and providing precursors for amino acids, nucleotides, and lipids, yet FDG-PET studies in patients demonstrate a wide variation in glucose uptake across tissues. Many explanations have been proposed for why certain cells take up so much glucose; however, none are consistent with all data.

Activation of growth signaling pathways, as well as pathways that enable hypoxia adaptation, can promote increased glucose metabolism. While this provides mechanistic insight into how cells adjust the metabolic network to enable this phenotype, it does not explain why it is beneficial. Perhaps increased glucose consumption in cancer is simply an epiphenomenon that derives from the link between growth signaling and glucose uptake, but the existence of this link argues that increased glucose metabolism benefits proliferating cells. Further, it is unclear whether the same benefits apply to non-proliferating cells with higher glucose uptake in the brain and immune system.

The most common explanation for increased glucose metabolism is that this satisfies an increased cellular demand for ATP. While undoubtedly true in some cases, this explanation is often unsatisfying. First, cells have access to many fuels that can be used to support ATP synthesis, yet glucose metabolism is particularly important for the proliferation and function of select cells. Second, in some cases there is minimal evidence that cells with high glucose uptake have greater energetic needs than other cells. In fact, ATP has been shown to be in excess for some cancer cells, and high glucose uptake is often linked to the lower ATP-yield process of fermentation. It has also been proposed that elevated glucose metabolism is important for macromolecule biosynthesis. While glucose can contribute to macromolecule synthesis, glucose carbon in all its fates is a minor contributor to cell mass, and the amount of glucose consumed by cells with high glucose uptake far exceeds the amount needed for any biomass components. Additionally, not all non-proliferating cells with high glucose uptake engage in increased biosynthesis.

To understand why select cells consume so much glucose, we need to get away from the same easy explanations and confront all the evidence. This means being open to new possibilities while also considering new experimental frameworks to test hypotheses. Admitting what we do not understand is the first step to unraveling this fundamental question around cell metabolism and tissue physiology.



**Kathryn Wellen**

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### **Metabolism and the epigenome**

There is a lot to be excited about in metabolism research, but I'll focus on two areas that we are incorporating into our work now. The first is the links between diet, systemic metabolism, and cancer. As a graduate student, I studied adipose tissue biology and systemic metabolic regulation and then became interested in cancer cell metabolism as a postdoc, and I've long been intrigued by the epidemiological evidence linking obesity and cancer. These links are now being studied by many labs on a mechanistic level. I think this area of research is important on the one hand because as we begin to understand how particular metabolic states or diets impact cancer development, there is the potential to empower people to make decisions that will decrease cancer risk. Another exciting concept is that strategies can be developed to optimize nutrition in specific ways to improve cancer therapeutic responses. Mechanistic studies using rodent models and engineered diets are providing important insights into diet-cancer links, but much more work is needed to understand how such findings can be translated to improve outcomes in humans. A second area that I personally am excited about, given our long-standing interest in understanding crosstalk between cell metabolism and the epigenome, is elucidating how metabolism is regulated distinctly in compartments, such as the nucleus. Of course, the compartmentalization of metabolism—both within membrane-bound organelles such as mitochondria and by multi-functional enzymes and multi-enzyme complexes—has long been appreciated, but studying how metabolite abundance and usage change in different compartments is challenging and associated with a number of caveats. Development of strategies to introduce internal standards prior to cellular fractionation, as well as rapid organelle purification methods, are facilitating new insights into compartmentalized metabolic processes. We are excited to apply such approaches to questions like how metabolites change in the nucleus under different conditions and the impact on chromatin modification and downstream biological processes.