**Metabolic decisions in development and disease—a Keystone Symposia report**

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**Abstract**

There is an increasing appreciation for the role of metabolism in cell signaling and cell decision making. Precise metabolic control is essential in development, as evident by the disorders caused by mutations in metabolic enzymes. The metabolic profile of cells is often cell-type specific, changing as cells differentiate or during tumorigenesis. Recent evidence has shown that changes in metabolism are not merely a consequence of changes in cell state but that metabolites can serve to promote and/or inhibit these changes. Metabolites can link metabolic pathways with cell signaling pathways via several mechanisms, for example, by serving as substrates for protein post-translational modifications, by affecting enzyme activity via allosteric mechanisms, or by altering epigenetic markers. Unraveling the complex interactions governing metabolism, gene expression, and protein activity that ultimately govern a cell’s fate will require new tools and interactions across disciplines. This report summarizes the Keystone eSymposium *Metabolic decisions in development and disease*, which brought together researchers in metabolism, development, and disease.

**Key words**: cell signaling, development, inborn errors of metabolism, metabolism, metabolome, stem cell differentiation

**Introduction**

While once regarded as a housekeeping process, metabolism is increasingly being appreciated as a driver for cell signaling and cell decision making. On March 24 and 25, 2021, experts in cell metabolism, developmental biology, and human disease met virtually for the Keystone eSymposium, *Metabolic decisions in development and disease.* The symposium brought together scientists exploring how metabolites impact cellular and developmental decisions in a diverse range of model systems investigating normal development, developmental disorders, dietary effects, and cancer-mediated changes in metabolism.

Several lines of evidence indicate that metabolites are not just passive building blocks for generating cellular biomass and energy. Speakers showed how metabolites can serve as active signaling molecules, providing a link between metabolic pathways and classic cell signaling pathways. For example, several metabolites can serve as substrates for protein and DNA modifications. Protein glycosylation depends on production of glycosyl donors like UDP-GlcNAc, acetyl-CoA is the acetyl donor for acetylation, and methylation depends on S-adenosylmethionine.1,2 In this way, metabolites can alter enzyme function, the epigenome, and gene expression.

Changes in metabolism are often associated with changes in cell state. Several speakers showed how metabolites can influence cell fate and differentiation. For example, the metabolite ɑ-ketoglutarate can promote epidermal stem cell differentiation by promoting DNA demethylation.3 In addition, H2O2 generated by metabolic pathways in the mitochondria is essential for epidermal and adipocyte stem cell differentiation.4,5 In disease, tumor cells must often change their metabolic phenotype to garner the nutrients needed to sustain continuous growth and cell division in an nutrient-deplete environment.6 Understanding these cell- and disease-specific metabolic profiles can lead to strategies that enrich or deplete for cells with specific properties of interest. For example, understanding metabolic differences between tumor and normal cells can reveal vulnerabilities within tumor cells while metabolic differences between pluripotent and differentiated cells can increase the efficiency of cellular reprogramming to create induced pluripotent stem (iPS) cells.

Finally, several speakers presented new techniques to investigate the metabolome. Studying the metabolome requires sophisticated computational techniques due to the huge number of metabolites, the fact that they can be generated via multiple pathways, and the complicated feedback loops that affect their production. Speakers presented new methods to interrogate protein-metabolite interactions, analyze metabolism at single-cell resolution, and integrate metabolomic, transcriptomic, and proteomic data.

The symposium served as an initial step in fostering collaborations across a range of disciplines and developing a conceptual framework for the roles of metabolites in biology and disease.

**Keynote Address**

*Warburg-like metabolism during vertebrate development*

**Olivier Pourquié** from Harvard Medical School gave the keynote presentation on the role of metabolism in determining cell fate during vertebrate development.Pourquié’s lab is interested in understanding the molecular and cellular processes that establish the cardinal features of the vertical body plan, such as an elongated body axis, segmentation, and bilateral symmetry. During their talk, Pourquié focused on the metabolic and signaling mechanisms important for axis elongation and segmentation in mouse embryos.

Vertebrate embryos develop in a head-to-tail fashion in which neuromesodermal precursors (NMP) are added to the posterior end of the presomitic mesoderm in a region called the tail bud or growth zone. As more cells are added, the presomitic mesoderm elongates, and the vertebrae and skeletal muscles eventually form.

Pourquié showed that signaling and metabolic gradients within the presomitic mesoderm drive segmentation and establish cell fate. For example, a fibroblast growth factor (FGF) and Wnt signaling activity gradient along the presomitic mesoderm defines cellular activity. Cells in the posterior region, where FGF and Wnt signaling is high, do not respond to periodic waves of the molecular oscillator segmentation Clock. Once cells are beyond the reaches of FGF and Wnt signaling, they are sensitive to the segmentation Clock and activate genes involved in segmentation.

Work in Pourquié’s lab has also identified a gradient of glycolytic activity parallel to the FGF/Wnt signaling gradient. Pourquié showed that FGF signaling creates the glycolytic gradient by influencing transcription of several glycolytic enzymes. Treating chicken embryos with FGF signaling inhibitors downregulated glycolysis by downregulating the expression of several rate-limiting glycolytic enzymes. Inhibiting glycolysis blocked axis elongation and presomitic mesoderm formation. To understand how glycolysis contributes to axis elongation, Pourquié’s group looked at the impact of glycolysis on NMPs. NMPs have the potential to differentiate into neural or mesodermal cells. Inhibiting glycolysis downregulated Wnt signaling and caused NMPs to differentiate toward a neural fate.7

Pourquié’s work demonstrates that the cells of the tail bud experience a type of metabolism that has been observed in cancer⎯Warburg metabolism.7,8 In cancer cells, Warburg metabolism is associated with an inverted pH gradient where the intracellular pH is higher than the extracellular pH.9 The tail bud also has an inverted pH gradient in which the posterior region has a more acidic extracellular pH and basic intracellular pH than anterior regions. This pH gradient is dependent on glycolytic activity.7,10 Pourquié showed that glycolysis likely affects intracellular pH via the production of lactate, which is transported out of the cell along with protons via the monocarboxylate transporter (MCT). Therefore, higher levels of glycolysis result in more basic pH.10

Working in human iPS cells, Pourquié’s group showed that the glycolysis-dependent intracellular pH gradient in the tail bud is important for differentiation. High pH favors non-enzymatic acetylation of beta-catenin, which promotes mesoderm induction.11 Pourquié showed that beta-catenin acetylation is dependent on glycolysis both *in vitro* and *in vivo.*12

Pourquié proposed a model (Fig. 1) by which regulation of intracellular pH controls Wnt signaling and ultimately cell differentiation in the presomitic mesoderm. In brief, FGF promotes glycolysis via transcription of glycolytic enzymes. Glycolysis increases lactate production, which promotes a more basic pH via the activity of MCT. The higher intracellular pH facilitates non-enzymatic acetylation of beta-catenin, which promotes Wnt signaling and leads to formation of the paraxial mesoderm from NMPs and favors axis elongation.

**Metabolic control of gene expression and developmental decisions**

*Metabolic and signaling crosstalk via the epigenome*

Crosstalk between metabolic and signaling pathways can occur via protein or nucleic acid modifications that rely on key intracellular metabolites. For example, glycosylation depends on production of glycosyl donors like UDP-GlcNAc while acetyl-CoA is the acetyl donor for acetylation.

**Kathryn Wellen** from theUniversity of Pennsylvania described how the cell can achieve specificity in metabolic regulation of the epigenome. Wellen asserted that the nucleus should be considered a distinct metabolic compartment and that spatio-temporal metabolite control within the nucleus may contribute to this specificity. For example, their lab has shown that ATP citrate lyase (ACLY), which cleaves citrate to produce acetyl-CoA, becomes activated in the nucleus upon DNA damage to promote DNA repair.13

Treating the nucleus as a distinct metabolic compartment requires methods to rigorously measure metabolites within the nucleus. Wellen’s group has developed an approach to subcellular acyl-CoA quantification dubbed stable isotope labeling of essential nutrients in cell culture with sub-cellular fractionation (SILEC-SF). In this method, cells grown in heavy media are combined with cells grown in normal media, lysed and fractionated. Metabolites are quantified via liquid chromatography/mass spectrometry (LC/MS). Wellen showed that SILEC-SF can successfully detect predicted compartment-specific changes in acyl-CoA abundance under hypoxic conditions.14,15 Wellen’s group plans to use SILEC-SF to understand the pathways through which acyl-CoAs are transported to or generated in the nucleus and how are these pathways mediate biological responses.

Wellen also described work in understanding the role of the hexosamine biosynthetic pathway, which UDP-GlcNAc, a substrate for glycosylation and GlcNAc modifications.1,16 Targeting the hexosamine biosynthesis pathway may be an effective strategy in pancreatic cancer.17–21

Wellen’s group has elucidated how nutrient deprivation, a feature of the pancreatic tumor microenvironment, impacts hexosamine synthesis. In pancreatic cancer cell lines, low glutamine levels decreased intermediate metabolites in the hexosamine biosynthesis pathway, but not UDP-GlcNAc. Wellen showed that while glutamine deprivation suppresses *de novo* hexosamine synthesis, cells can generate UDP-GlcNAc via the hexosamine salvage pathway via the turnover of O-GlcNAc protein modifications or breakdown of glycans. Tumor cells overexpress components of the salvage pathway, such as N-acetyl-D-glucosamine kinase (NAGK). Knocking out *NAGK* elevated *de novo* synthesis of UDP-GlcNAc and impaired xenograft tumor growth in mice, consistent with the idea that NAGK and hexosamine salvage become more important as tumors grow and the tumor environment is more nutrient restricted.22

Wellen proposed that under high-nutrient conditions (Fig. 2) , cells can toggle between *de novo* hexosamine biosynthesis and salvage. Under nutrient deprivation, *de novo* biosynthesis is suppressed, and cells shift toward NAGK-dependent hexosamine salvage.22 Recent work in an independent lab has also found an important role for hexosamine salvage in pancreatic cancer.23 This work suggests that nuclear-cytosolic recycling pathways can play crucial roles under conditions of nutrient stress.

*Metabolic changes and stem cell differentiation*

Metabolic changes can contribute to changes in cell fate by serving as co-substrates for chemical modifications that impact gene expression or by influencing signaling networks and modifications that are important for cell fate and cell identity. Understanding cell-type specific metabolic profiles at different stages of development or disease could help develop strategies that enrich or deplete for cells with specific properties of interest.

**Lydia Finley** fromMemorial Sloan Kettering Cancer Center presented work on the effect of metabolic changes on cell fate during embryonic development. Finley’s work illustrates that metabolic perturbations can drive signal transduction networks important for cell fate. They argued that metabolism should be considered co-equal to cell signaling in regulating cell identify.

Finley described work in two types of mouse embryonic stem cells (ESC): naive ESCs, which represent the earliest pluripotency state that can be captured, and metastable ECSs, which have a more committed phenotype.

Finley showed that naïve cells shuttle more glucose-derived carbons and fewer glutamine-derived carbons into the TCA cycle than metastable cells. This metabolic difference had functional consequences as, similar to most cells, metastable ESCs could not proliferate under glutamine deprivation but naive ESCs could.24 Glutamine-independent proliferation was associated with higher NANOG expression, a marker of higher self-renewal. In the more heterogenous metastable ESCs, removing glutamine shifted the population toward a more self-renewing state by killing the most committed cells.24,25 Finley noted that this can be used to increase the efficiency of reprogramming somatic cells to pluripotency. Subjecting reprogrammed cells to a pulse of glutamine deprivation eliminated incompletely reprogrammed cells, leaving a more pluripotent population.25

Finley described how naive ESCs survive in the absence of glutamine. Glutamine is produced using the carbon backbone of ɑ-ketoglutarate. In naive cells, high levels of ɑ-ketoglutarate promote DNA demethylation, which is associated with self-renewal in ESCs.24,25

While DNA methylation is associated with cell differentiation in ESCs, decreased methylation at lineage-specific loci is critical for differentiation in adult stem cells.26,27 Finley showed that ɑ-ketoglutarate can also tune demethylation in adult epidermal stem cells and thus promote differentiation (Fig. 3) . Depriving epidermal stem cells of serine, a critical donor for methylation, reduced histone methylation and induces differentiation. Finley showed that production of ɑ-ketoglutarate via the serine synthesis pathway drives demethylation and cell differentiation, not lack of serine itself. In an allograft model, tumors were sensitive to serine starvation, demonstrating how targeting metabolic pathways to control cell fate can lead to anti-cancer strategies.3 Together, these results support a model by which ɑ-ketoglutarate can exert different effects by facilitating demethylation programs that reinforce or change cell fate.

*Measuring glycolytic oscillations in the presomitic mesoderm*

**Alexander Aulehla** from the European Molecular Biology Laboratory presented work on the role of glycolytic flux in mouse embryo segmentation. In the developing vertebrate embryo, segmentation is a result of oscillatory activity of the somite segmentation clock, which consists of signaling molecules such as Notch, Wnt, and FGF. With regard to metabolism, there is a glycolytic gradient along the vertebrate presomitic mesoderm, with higher glycolytic activity at the posterior end.7 Aulehla’s lab has developed a method to measure dynamics in glycolytic activity *in vitro* using a pyruvate-FRET-sensor.28,29 They showed that the *de novo* formation of the PYRATE-FRET ratio gradient was linked to presomitic mesoderm differentiation.8

As Pourquié described, the presomitic mesoderm contains a signaling gradient, with higher FGF and Wnt activity at the posterior end, as well as a metabolic gradient, with higher glycolytic activity at the posterior end. Aulehla is interested in a third feature of the presomitic mesoderm, namely, the oscillatory activity of the segmentation clock. They described unpublished work investigating the molecular mechanisms behind signaling oscillations and periodicity, the role of glycolysis in controlling segmentation clock oscillations, and in determining whether metabolic oscillations exist in the presomitic mesoderm.

*Stress-induced changes to lipid metabolism*

During development, environmental stresses can impact the resulting phenotype of an organism. **Alex Gould** from the Francis Crick Institutepresented work on how developing animals cope with and respond to stresses in their environment. To answer these questions, Gould’s group is developing *Drosophila* models to investigate different types of development stress, such as nutrient restriction, hypoxia, and diet. During their talk, Gould focused on the role of lipid metabolism in the developing central nervous system (CNS) and renal system.

Gould’s lab has showed that glia play an important role in activating neuroblast growth within the neural stem cell niche in *Drosophila* larva. Under hypoxia, neuroblast divisions are protected by lipid droplets produced in the glial niche.30,31 Knocking down the enzyme DGAT1, which produces triacylglycerol, a key component of these lipid droplets, increased lipid peroxidation and decreased neural stem cell proliferation.31 While Gould’s work suggests a beneficial role for lipid droplets in sustaining the proliferation of the stem cell lineages, other studies in other contexts have reported opposite effects.

To investigate the role of biological context on the impact of stress-induced lipid droplets, Gould’s group turned to the *Drosophila* renal system. In mammals, chronic kidney disease (CKD) is associated with accumulation of lipid droplets in the nephron and can be recapitulated in mice by a high-fat diet (HFD). In flies, a HFD results in lipid droplets in the nephrocyte, the filtering unit of the renal system, along with morphologic and functional impairments, including a decrease in mitochondrial volume and compromised endocytosis of small and large dextrans. Overexpressing ATGL, a lipase that cleaves triacylglycerol and thus disrupts lipid droplets, rescued these phenotypes. Gould put forth a model wherein flux through the lipid droplet protects renal endocytosis from lipotoxicity. Normally, excess lipids circulating in the fly hemolymph are endocytosed by nephrocytes and end up in lipid droplets via the activity of DGAT1. However, when the system is overwhelmed with lipids, such as in the case of a HFD, the lipids go through a yet-to-be determined non-lipid droplet route, which is toxic to the mitochondria.32

Broadly speaking, Gould’s work shows that stress-induced lipid droplets appear to play similar roles in these two contexts, ie, the neural stem cell niche and nephrocytes, in which fatty acid flux through triacylglycerol-containing lipid droplets minimizes lipid peroxidation and protects cell function.

*Metabolite sensing via protein post-translational modifications*

Many post-translational modifications, such as methylation, acetylation, and lipidation, are formed by metabolites. Often, these covalent modifications are catalyzed by enzymes; however, in many cases the concentration of the metabolite itself can be rate limiting.

**Aurelio Teleman** from Deutsches Krebsforschungszentrum presented work on how metabolite-induced protein post-translational modifications can affect signaling pathways.2 Teleman focused on an example of metabolite sensing in which the lipid stearic acid regulates cell signaling via post-translational modification. Stearic acid has been associated with mitochondrial fusion and activation and inhibition of oncogenic signaling. It is found in the diet and can also be formed from palmitic acid via the enzyme Elovl6.Teleman showed that in flies, *Elovl6* knockout causes lethality if flies are fed a small amount of a mitochondrial inhibitor, suggesting that lack of stearic acid sensitizes flies to mitochondrial inhibition. *Elovl6* mutant flies had defective mitochondria that were unable to fuse in the absence of stearic acid. Similar defects in mitochondrial fusion were seen in HeLa cells grown in delipidated medium. This phenotype was rescued with addition of stearic acid.33

Teleman showed that stearic acid can covalently attach to the transferrin receptor on the cell membrane. Normally, the transferrin receptor activates ubiquitination and degradation of mitofusin via JNK signaling. When modified with stearic acid, however, the transferrin receptor is unable to activate JNK, and therefore mitofusin remains active and able to fuse mitochondria.33

This mechanism is biologically relevant both in flies and in humans. Flies fed a diet lacking stearic acid had speckled/unfused mitochondria, which resolved with a stearic acid-containing diet.33 In humans, a double-blind, randomized, crossover study showed that eating a low stearic acid diet resulted in fragmented mitochondria in white blood cells. Within three hours of eating a high stearic acid meal, the proportion of fragmented mitochondria decreased while the proportion of fused mitochondria increased.34

Teleman’s group is using mass spectrometry to identify other proteins modified by stearic acid. In addition to the transferrin receptor, GNAI proteins are also modified upon consumption of stearic acid. GNAI proteins can be modified with palmitic acid or stearic acid in a competitive manner that depends on the lipid levels in the cell. When modified with palmitic acid, GNAI is able to activate EGFR signaling. When modified with stearic acid, it cannot. (Nuskova, in revision)

Teleman’s work shows that metabolites present in food, like stearic acid, can have direct effects on cell signaling via post-translational modifications. In addition, they stressed the need to shift the current thinking of lipid modification from a binary on/off switch to understand the differential effects of specific modifications.

*Short talk: tryptophan metabolism in cell fate*

**William Tu** from Kathrin Plath’s lab atUCLApresented unpublished work on the role of tryptophan metabolism in cell fate decisions and development. Plath’s lab is broadly interested in understanding what regulates how cells change from one type to another, with implications in normal development and disease. Specifically, they have focused on reprogramming differentiated cells to iPS cells. While genomic studies have revealed many insights on the genomic and molecular features of this process, less is known about the role that metabolism may play. Tu is investigating whether specific metabolites regulate cell fate transition and identifying the mechanisms behind metabolic control of cell fate transition. They showed how dietary nutrients can affect cell fate transition and how these insights can be leveraged to make induced pluripotency more efficient and accurate.

**Metabolic communication across cells and tissues**

*Inter-organ metabolic crosstalk in Drosophila*

**Irene Miguel-Aliaga** from Imperial College Londonpresented work on understanding metabolic crosstalk between organs in *Drosophila*, specifically between the gut and other organs. Gut neurons, hormones, microbes, immune cells, and metabolites communicate with other organs and regulate food intake and energy balance

Flies have a relatively complex gastrointestinal system with similar cell types to those found in humans, including intestinal stem cells (ISC), epithelial cells, and enterocytes. Prior work in Miguel-Aliaga’s lab revealed that the guts of male and female flies show striking differences at multiple levels, including gene expression and physiological features. Initially, their group focused on sex-related differences in ISCs. In female flies, ISCs divide more rapidly. This allows females to resize the gut during reproduction but also makes them more vulnerable to gastrointestinal tumors.3535,36

Miguel-Aliaga’s group has been systematically characterizing sex differences in different cell types of the fly gut. During their talk, they focused on sex differences in enterocytes. Miguel-Aliaga showed that enterocytes in the posterior region of the male fly gut had higher expression of genes involved in multiple stages of carbohydrate metabolism, including starch digestion, glucose transport, glycolysis, and the pentose phosphate pathway. A FRET-based metabolite assay confirmed that these transcriptional differences correlated with higher glucose levels in males.37

In their ISC work, Miguel-Aliaga’s group found that the cell’s intrinsic sexual fate was important for sex-specific proliferation capacities; however, intrinsic factors did not play a role in sex-related metabolic differences in enterocytes. Masculinizing or feminizing enterocytes did not affect the sexual dimorphism seen in enterocytes, suggesting that it was controlled by external factors.35,37

The region of the gut that displays metabolic sexual dimorphism is adjacent to the testes. Miguel-Aliaga showed that this proximity enables metabolic crosstalk between the two organs. Release of the testis cytokine by the testes activates Jak/Stat signaling in male gut enterocytes and results in regional upregulation of carbohydrate genes. This has effects on behavior⎯the degree of masculinization in enterocytes is associated with food intake. Abrogating the male bias in carbohydrate metabolism reduced food intake while increasing carbohydrate metabolism via ectopic cytokine signaling or gene expression increased food intake. By downregulating metabolic genes and observing the effect on food intake, Miguel-Aliaga’s group showed that enterocytes control food intake by secreting citrate. Reducing intestinal citrate secretion reduced citrate levels in testis somatic cells, which was found to be important for sperm production.37

Miguel-Aliaga put forth a model in which the testes secrete testis cytokine to increase glucose metabolism in enterocytes, which in turn increase food consumption and secrete citrate, which is used by the testes to produce sperm. Their group is interested in investigating whether there are other examples of metabolic communication between adjacent organs.

*Metabolic crosstalk between tumors and peripheral organs*

**Norbert Perrimon** fromHarvard Medical Schoolpresented research on inter-organ communication to understand organ wasting in *Drosophila.* Perrimon’s group takes a system-level approach to understand communication between different organs by identifying the hormones that mediate communication and how organs coordinate the use and storage of nutrients, particularly in response to changes in diet, obesity, and disease.

Over the past 10 years, Perrimon’s group has characterized the communication between different organs, establishing a network of signals between organs.38–48 During their talk, Perrimon focused on organ-wasting model in cancer models in fruit flies.43

Perrimon showed that tumors secrete several factors that affect nutrient utilization in peripheral organs. For example, ImpL2 is released by tumors into the circulation where it can limit nutrient utilization in the muscle, leading to lipid loss, muscle wasting, hyperglycemia.43 Another factor released by tumors, UPD3, contributes to wasting by blocking insulin signaling. Finally, tumors secrete Pvf1, which activates ERK signaling in peripheral tissues and actively promotes tissue degradation and nutrient release.

Perrimon described work to systematically identify inter-organ communication factors and analyze their system-wide effects using snRNAseq and proximity labeling. They showed how snRNA-seq can be applied to generate full-body global maps to identify inter-organ communication factors and generate hypotheses about the pathways that are dysregulated in different cell types. Perrimon also presented unpublished work investigating the intracellular signaling pathways in peripheral tissues that are activated by tumors and lead to organ wasting.

*Integrating signals from the microbiome and circadian clock to affect lipid metabolism*

**Lora Hooper** fromThe University of Texas Southwestern Medical Centerpresented their work on how signals from the microbiome and circadian clock converge to regulate lipid metabolism in the gut. The intestinal microbiota can influence mammalian metabolism via several ways. The microbiota can break down dietary components like polysaccharides into simple sugars that are more easily absorbed. Hooper’s work shows that the interactions can be more complicated as well. Their work demonstrates how gut microbiota can regulate the circadian clock in gut epithelial cells to impact lipid absorption and metabolism and ultimately fat storage and body composition.

It has been known that the gut microbiota can impact fat storage and body composition. Germ-free mice have lower body fat percentage and are protected from the effects of a HFD on weight and body composition.49

To illustrate how the microbiota affect fat storage, Hooper focused on the role of two proteins in gut epithelia cells: Nfil3, a transcription factor regulated by the circadian clock, and HDAC3, a histone deacetylase that impacts lipid absorption and metabolism.

Hooper showed that epithelial *Nfil3* expression exhibits circadian rhythms that are dampened in the absence of a gut microbiome. Knocking out *Nfil3* from gut epithelial cells in mice protected them from HFD-induced obesity and reduced lipid absorption in the gut. Transcriptomics analysis of *Nfil3* knock-out mice showed that Nfil3 regulates a circadian metabolic gene transcription program centered on fatty acid metabolism. Altogether, Hooper’s work shows that microbiota trigger circadian expression of epithelial Nfil3, which regulates expression of CD36, the long-chain fatty acid transporter responsible for lipid uptake. The effect of microbiota on Nfil3 expression is indirect—interactions between the microbiota and sub-epithelial immune cells stimulate Nfil3 expression via activation of IL-22R. Inside the epithelial cell, activation of IL-22R activates STAT3, which downregulates Rev-erbalpha, a component of the circadian clock and a repressor of Nfil3 expression.50,51

The second part of Hooper’s talk focused on another protein that integrates signals from the microbiome and circadian clock to impact lipid metabolism: HDAC3.52 Classically, HDAC3 is a histone deacetylase that regulates chromatin accessibility. Hooper showed that it can have other roles as well. The microbiota impacts HDAC3 expression. In germ-free mice, HDAC3 expression is downregulated. Similar to *Nfil3* knock out, knocking out *HDAC3* reduced lipid absorption, lowered body fat percentage, and protected them from the effects of a HFD. Hooper showed that HDAC3 can also affect expression of CD36. Knocking out *HDAC3* repressed the circadian expression of CD36. Briefly, HDAC3 binds to the CD36 promoter in a circadian manner, with high binding at night and low binding during the day. This rhythmic binding is abrogated in germ-free mice.

Hooper’s work shows that there are multiple systems that can converge on the same lipid metabolic pathway. Their lab is trying to tease out this complexity by determining the effects of specific gut bacteria on lipid metabolism pathways.

*Towards genome-scale personalized metabolic networks*

**AJ Marian Walhout** from theUniversity of Massachusetts Medical Schoolpresented work on developing personalized metabolic networks in *C. elegans.* Walhout’s lab is broadly interested in the effects of nutrients on gene expression and physiology and how metabolism and gene expression interact at a system level. The ultimate goal is to integrate nutrigenetics, genomics, and transcriptomics to understand how diet affects individuals, predict health outcomes, and develop personalized therapeutic interventions.

Walhout’s group has developed the first genome-scale *C. elegans* metabolic network that incorporates approximately 1200 genes, 600 enzymes, 2000 reactions, and 900 metabolites.53 The model was recently updated to include more genes and scRNAseq data to predict tissue-relevant metabolism at the network, pathway, reaction, and metabolite levels.54 Researchers can access this metabolic model at <http://wormflux.umassmed.edu/>.

Walhout’s group is now working on developing personalized metabolic network models from various *C. elegans* strains. They presented unpublished data from a collaboration with Erik Andersen at Northwestern University and Frank Schroeder at Cornell to relate differences in metabolites between strains with sequencing and transcriptomics data.

*Short talk: Developing tools to profile the secretome*

**Wei Wei** from Jon Long’s lab atStanford Universitypresented work on developing tools to profile the secretome *in vivo.* Wei has developed three separate tools to characterize three types of secretion: conventional secretion, in which peptides are secreted via vesicles transported from the Golgi body; nonconventional secretion, in which peptides are secreted from the cytosol; and ectodomain shedding, in which membrane proteins are cleaved from the cell surface. In each of these approaches, a genetically labeled enzyme that biotinylates proteins is incorporated into the cells of interest. Biotinylated proteins are captured from the target tissue and identified via mass spectrometry. To map conventional secretion, Wei targets the enzyme TurboID to the ER-Golgi, while a cytosolic TurboID is used to map unconventional secretion. A different biotinylating enzyme, subtiligase, is anchored to the membrane to map ectodomain shedding. Wei showed that these tools can capture cell-type selective secretome markers while also revealing new insights into protein secretion. For example, cytosolic TurboID in hepatocytes in mice revealed that a sugar-rich diet causes unconventional secretion of BHMT, an enzyme involved in methionine metabolism that had not previously been known to be secreted.55 This set of tools enables researchers to directly measure secreted peptides from a known cell of origin *in vivo*.

**Maintenance and perturbation of metabolic networks**

*Single-cell metabolomics profiling*

**Theodore Alexandrov** fromEMBL and UCSD presented work onspatial and single-cell metabolomics. Alexandrov’s lab has developed a method, SpaceM, that provides single-cell metabolomics information. In brief, cells grown in culture are fixed and visualized via microscopy. This provides information on cell image, morphology, and spatial relationships between cells. The slides are then analyzed via MALDI-imaging mass spectrometry, in which a laser scans the sample and generates a mass spectrum for every point in the sample. With the tool Alexandrov uses, each point is approximately 5 to 10 μm, and spectra contain information on over 100 molecules, including metabolites, lipids, and drugs. This method generates a wealth of data, including mass spectra intensities of hundreds of metabolites, lipids, small molecules; fluorescence intensities; and morphometric properties for every cell in the sample. By overlaying the microscopy image with the mass spectrum image, SpaceM can identify the metabolites present in a given cell. The method is fairly high throughput and is amenable to various cell types.56 Alexandrov presented unpublished data showing how SpaceM can be used to reveal co-existing metabolic states within a sample and delineate how these populations change due to external factors.

*Metabolic crosstalk between tumor cells and host cells*

**Ayelet Erez** from the Weizmann Institute of Science presented work on understanding cross talk via tumor and host via amino acids. Previous work in Erez’s lab has shown that the urea cycle is dysregulated in cancer cells. The urea cycle normally converts excess nitrogen in the form of ammonia to urea, which is excreted in the urine. While the complete urea cycle occurs in the mitochondria and cytosol of hepatocytes, most healthy tissues express some urea cycle enzymes. In cancer cells, urea cycle enzymes are dysregulated to increase availability of nitrogen-rich compounds for synthesis of pyrimidines and amino acids. These excess nutrients support cancer growth and promote mutagenesis, ultimately leading to poorer outcomes.57–59

During this work, Erez’s group also noticed changes in the urea cycle in the liver. Mice with various types of cancer had lower expression of all urea cycle enzymes, which correlated with lower urea levels in the urine. In addition, human patients with cancer have lower urea levels in the urine compared to matched controls, suggesting that urea cycle dysregulation may be a common feature of many cancers.58

Erez showed unpublished work demonstrating crosstalk between tumor cells and normal hepatocytes that affects urea cycle activity in hepatocytes and tumor cell growth and proliferation.

*Inferring metabolomes from proteomes*

While the metabolome is a product of the proteome, the complicated network of metabolic enzymes means that inferring a cell’s metabolome based on the proteome is not straightforward.

**Markus Ralser** from The Francis Crick Institutepresented research on understanding the connectivity from genome to proteome to metabolome. Their talk focused on using metabolic perturbations to derive the logic of metabolism.

Many cells grown in culture have been genetically modified so that they lack essential metabolic genes. While this is not an issue for many applications as cells can be grown in nutrient-rich media, it makes it difficult to study metabolism. Ralser’s group has created thousands of metabolically competent yeast strains in which many of the metabolic genes have been restored. Using these strains, their lab has developed high-throughput methods for cell cultivation, metabolite extraction, and targeted metabolomics.60 One of the first applications of this method was to conduct genome-spanning scans looking for genes that affect amino acid metabolism. By systematically deleting each gene in the yeast genome and looking at the effect on amino acid metabolism, Ralser’s group has linked each yeast gene to a metabolic phenotype.61 This has also revealed some novel roles for amino acids. For example, Ralser showed that yeast cells can actively take up lysine, which can make them more tolerant to oxidative stress.62

Amino acid metabolism occursnot only within cells but also between cells. Yeast can form self-establishing communities in which cells share metabolites.63 Ralser showed that this cooperative metabolism increases efflux activity and may provide benefits beyond metabolite accessibility.

Ralser’s group is also working on high-throughput methods to measure proteomes. Their group has used SWATH-MS to measure hundreds of proteomes and link them to metabolomes. By associating changes in enzyme levels to changes in associated metabolites, they can develop a predictive model to predict metabolite concentration based on the proteome.64They have recently modified their proteomics platform to scale up from hundreds to thousands of proteomes.65–67

*Elucidating tissue-specific lipogenesis pathways*

**Joshua Rabinowitz** from Princeton University presented work on delineating the mechanisms of *de novo* lipogenesis in the liver and adipose tissue. *De novo* lipogenesis is a hallmark of non-alcoholic fatty liver disease, which can result from excess fat as well as excess sugar in the diet. Synthesizing fat from carbohydrates requires both a carbon source as well as the reductant NADPH. Rabinowitz focused on how different tissues generate NADPH for lipogenesis. In mammalian cells, there are three major pathways for NADPH production: the oxidative pentose phosphate pathway, and the activity of malic enzyme and IDH1 in the TCA cycle. Work in Rabinowitz’s lab using an *in silico* metabolic model predicted that NADPH may also be produced by folate-mediated serine catabolism.68

Rabinowitz presented unpublished data on determining the carbon and NADPH sources for *de novo* lipogenesis in different tissues. Rabinowitz hopes that understanding the pathways involved in metabolism, and particularly those involved in pathology, such as liver *de novo* lipogenesis in fatty liver disease, can reveal actionable targets for therapeutics.

*Short talk: Visualizing metabolic effects of oncogenic mutations*

**Anupama Hemalatha** from Valentina Greco’s lab at Yale University presented work on understanding metabolic changes induced by oncogenic mutations. Greco’s lab has characterized two cases of oncogenic tolerance in mouse skin in which skin cells acquire oncogenic mutations without developing any oncogenic phenotype. In the first case, a gain-of-function mutation in beta-catenin is eliminated in the skin as mutant cells are outcompeted via selective differentiation. In the second case, cells acquire constitutive activation of HRAS and are not outcompeted by wild-type cells.69,70

Hemalatha presented unpublished work that combines live mouse skin imaging with optical redox ratio imaging developed in Melissa Skala’s lab to monitor how these oncogenic mutations affect metabolic activity throughout the epidermis *in vivo*.

*Short talk: miR-1 targets in muscle physiology*

**Paula Gutierrez-Perez** from Luisa Cochella’s lab at the Research Institute of Molecular Pathologypresented unpublished work on understanding how miR-1 sustains muscle physiology. miR-1 is a deeply conserved muscle-specific miRNAthat acts as transcriptional repressors by mediating mRNA degradation.Knock-out studies reveal that miR-1 is important in several steps of muscle development.71,72 While several putative downstream targets of miR-1 have been proposed, none are conserved. Gutierrez-Perez showed that miR-1 can control V-ATPase complex assembly, which has implications for mitochondrial function and proteostasis in the muscle.

**Metabolic disorders**

*Investigation inborn errors of metabolism*

**Ralph DeBerardinis** from the University of Texas Southwestern Medical Centerpresented work on how genetic mutations result in disease through their effects on metabolism. During the presentation, they focused on work on inborn errors of metabolism, rare genetic disorders caused by mutations in metabolic enzymes that interfere with growth and development. DeBerardinis is part of a clinical program of over 800 subjects at UT Southwestern that uses metabolomics and genomics to identify candidate genes in patients with inborn errors of metabolism.

DeBerardinis described one patient from the program who had epilepsy and neurodevelopmental disabilities of unknown molecular cause. Metabolomics analysis revealed a previously unseen pattern in their metabolomic profile that consisted of high lactate, proline, alanine, and glutamate levels. Genetic sequencing revealed variants in *LIPT1*, which encodes lipolytransferase-1 and is important for TCA cycle activation. DeBerardinis noted that while the functional significance of the sequencing data was not immediately clear, when paired with the metabolic phenotype, the mechanism became more apparent.73 Their group is now working on functional assays of *LIPT1* variants to see what effect they have on lipolytransferase activity and metabolism.

DeBerardinis also presented work to investigate the effects of inborn errors on development. There are several examples of mutations in metabolism enzymes affecting development. For example, newborns with inborn errors in pyruvate dehydrogenase often exhibit defects in the corpus collosum in the brain.74 While it is unclear why these defects occur, it demonstrates that specific pathways are important for different aspects of development. DeBerardinis’s group has developed a system to assess metabolic properties during gestation in developing embryos and placenta in mice. They presented unpublished work showing how this method can be used to better understand the impact of patient-derived genetic variants on metabolism during development *in utero.*

*Mitochondria as signaling organelles*

**Navdeep Chandel** fromNorthwestern University presented work on understanding the mitochondria as a signaling organelle. While many learn about mitochondria as the powerhouse of the cell, Chandel argued that their bioenergetic and biosynthetic functions are not strictly required in many cells as the TCA cycle can provide almost all the metabolites needed. Chandel showed that in stem cells, one of the main roles of mitochondria is to determine cell fate and function.

Mitochondria generate a variety of signals that can control stem cell fate and function, including H2O2, NAD/NADH, fumarate, and succinate. Mitochondrial H2O2  is necessary for adipocyte differentiation from human mesenchymal stem cells4 as well as epidermal stem cell differentiation.5 Chandel recently published a model for how reactive oxygen species may control stem cell fate. They argued that there is a physiological role of mitochondria to produce H2O2 to drive normal differentiation. Overproduction of H2O2 can lead to stem cell exhaustion and depletion while underproduction or exposure to antioxidants can cause stem cells to undergo cell death.75

Chandel’s group has also investigated the role of mitochondria in hematopoietic stem cell (HSC) differentiation. They showed that mitochondria metabolism can affect HSC differentiation by affecting DNA and histone methylation. Shutting down mitochondria in HSCs resulted in increased NADH/NAD levels, and increased DNA and histone methylation, which ultimately inhibited differentiation.76

Finally, Chandel presented unpublished work on understanding the role of mitochondria in lung alveoli development.

*A systematic approach to protein-metabolite interactions*

**Jared Rutter** from the University of Utah presented work on identifying protein-metabolite interactions**.** Several years ago, Rutter’s lab was involved in discovering the identity of the mitochondrial pyruvate carrier (MPC), the protein complex necessary and sufficient for uptake of pyruvate into the mitochondria.77 Since then, the group has used the MPC as a tool to understand the effects of preventing or inducing mitochondrial pyruvate entry. Rutter’s group has found that pyruvate transport can have profound effects on stem cell homeostasis and differentiation, oncogenesis, and cardiomyocyte size.78–81

Rutter’s lab has been trying to understand the metabolic and signaling mechanisms that explain how mitochondrial pyruvate entry, which does not have profound metabolic consequences, can have profound effects on transcription and cell decisions. One of the major challenges to this is the lack of sensitive, reliable methods to investigate protein-metabolite interactions in a systematic way. If metabolites are playing signaling roles, they are likely to do so by interacting with proteins. Rutter’s group has developed mass spectrometry integrated with equilibrium dialysis (MIDAS) to address this need. MIDAS is a screening platform to systematically discover protein-metabolite interactions. In brief, purified proteins are separated from a pool of metabolites via a dialysis membrane. While the metabolites are able to freely diffuse across the membrane, the protein is restricted to one side of the chamber. Metabolites in the two chambers are quantified by mass spectrometry. Those that bind to the protein will be enriched in the protein-containing chamber.82

Rutter showed unpublished work using MIDAS to characterize protein-metabolite interactions for hundreds of proteins involved in metabolic pathways or growth factor signaling. Their group is collaborating with other labs to characterize these interactions, including biochemical analyses to investigate the effect of metabolite interactions on enzyme function and structural analyses to characterize the binding interaction.

*Dietary protein composition and metabolism: the role of methionine*

**Jason Locasale** from Duke University presented work on the role of methionine metabolism on cell phenotype. Locasale’s group is broadly interested in three main areas: developing quantitative and computations technologies to understand metabolic pathway regulation, delineating how metabolism influences chromatin status, and understanding how nutrition influences metabolic pathways in health and cancer.

Environmental influences, like diet, can link metabolites to chromatin accessibility, primarily by providing substrates involved in chromatic modification. These changes have transcriptional effects that may ultimately lead to differences in cellular state.83 Locasale showed that changes in metabolism may be associated with diseases like cancer via their effects on chromatin. While few metabolic pathway mutations are oncogenic, there are several examples of chromatin modifications downstream of metabolic pathways associated with cancer.84

Locasale focused on methionine metabolism, which processes the carbon unit for methylation. Methionine concentration in the plasma is highly variable.85 Most of the variability comes from the diet. While many people understand that the type of sugar or fat in one’s diet can have differential effects on metabolism, there is limited research on the effect of dietary protein composition on metabolism.

In animal studies, dietary methionine can affect lifespan and body weight.86 Locasale showed that methionine concentrations can range 3 to 4 fold in different types of diet, which can lead to differences in methionine uptake and methionine concentrations. Locasale’s group has characterized the effects of methionine restriction on metabolism in mice, which suppresses metabolites of the methionine cycle.87 They also showed that methionine metabolism can influence histone methylation, thus linking methionine to chromatin dynamics and potentially changes in cellular state.85

*Short talk: Mitochondrial defects and premature aging*

**Juan Landoni** from Anu Suomalainen-Wartiovaara’s lab at the University of Helsinki presented research on the role of mitochondria in premature aging. Many murine models replicate the process of accelerated aging observed in human diseases by promoting stem cell defects via nuclear genome instability. One model, however, the mitochondrial DNA (mtDNA) mutator mouse, contains defects in the polymerase responsible for mtDNA replication (PolG).88–91 mtDNA mutator mice exhibit features of accelerated aging, including stem cell defects and early death. This model has been used to support the mitochondrial theory of aging, which proposes that accumulation of mtDNA mutations leads to aging. Landoni has characterized the mtDNA mutator mouse model. They showed that mtDNA mutator iPSCs exhibited delays in cell proliferation and cell cycle progression as well as nuclear DNA damage. Landoni showed that defects in PolG increase the replication rate of mtDNA, causing cells to preferentially distribute dNTPs to the mitochondria over the nucleus. This leads to dNTP deficiency in the nucleus, replication stalling, and DNA damage. Landoni’s work shows that, instead of providing support for the mitochondrial theory of aging, the mtDNA mutator mouse model actually induces accelerated aging via similar mechanisms as other models, ie, via nuclear genome instability.92

**Acknowledgements**

**Competing Interests**

**References**

1. Campbell S.L. & K.E. Wellen. 2018. Metabolic Signaling to the Nucleus in Cancer. *Mol. Cell* **71**: 398–408.

2. Figlia G., P. Willnow & A.A. Teleman. 2020. Metabolites Regulate Cell Signaling and Growth via Covalent Modification of Proteins. *Dev. Cell* **54**: 156–170.

3. Baksh S.C., P.K. Todorova, S. Gur-Cohen, *et al.* 2020. Extracellular serine controls epidermal stem cell fate and tumour initiation. *Nat. Cell Biol.* **22**: 779–790.

4. Tormos K.V., E. Anso, R.B. Hamanaka, *et al.* 2011. Mitochondrial complex III ROS regulate adipocyte differentiation. *Cell Metab.* **14**: 537–544.

5. Hamanaka R.B., A. Glasauer, P. Hoover, *et al.* 2013. Mitochondrial reactive oxygen species promote epidermal differentiation and hair follicle development. *Sci. Signal.* **6**: ra8.

6. Kalyanaraman B. 2017. Teaching the basics of cancer metabolism: developing antitumor strategies by exploiting the differences between normal and cancer cell metabolism. *Redox Biol.* **12**: 833–842.

7. Oginuma M., P. Moncuquet, F. Xiong, *et al.* 2017. A Gradient of Glycolytic Activity Coordinates FGF and Wnt Signaling during Elongation of the Body Axis in Amniote Embryos. *Dev. Cell* **40**: 342-353.e10.

8. Bulusu V., N. Prior, M.T. Snaebjornsson, *et al.* 2017. Spatiotemporal Analysis of a Glycolytic Activity Gradient Linked to Mouse Embryo Mesoderm Development. *Dev. Cell* **40**: 331-341.e4.

9. Webb B.A., M. Chimenti, M.P. Jacobson, *et al.* 2011. Dysregulated pH: a perfect storm for cancer progression. *Nat. Rev. Cancer* **11**: 671–677.

10. Oginuma M., Y. Harima, O.A. Tarazona, *et al.* 2020. Intracellular pH controls WNT downstream of glycolysis in amniote embryos. *Nature* **584**: 98–101.

11. Hoffmeyer K., D. Junghans, B. Kanzler, *et al.* 2017. Trimethylation and Acetylation of β-Catenin at Lysine 49 Represent Key Elements in ESC Pluripotency. *Cell Rep.* **18**: 2815–2824.

12. Diaz-Cuadros M., D.E. Wagner, C. Budjan, *et al.* 2020. In vitro characterization of the human segmentation clock. *Nature* **580**: 113–118.

13. Sivanand S., S. Rhoades, Q. Jiang, *et al.* 2017. Nuclear Acetyl-CoA Production by ACLY Promotes Homologous Recombination. *Mol. Cell* **67**: 252-265.e6.

14. Trefely S., C.D. Lovell, N.W. Snyder, *et al.* 2020. Compartmentalised acyl-CoA metabolism and roles in chromatin regulation. *Mol. Metab.* **38**: 100941.

15. Trefely S., K. Huber, J. Liu, *et al.* 2020. Quantitative sub-cellular acyl-CoA analysis reveals distinct nuclear regulation. *bioRxiv* 2020.07.30.229468.

16. Wellen K.E., C. Lu, A. Mancuso, *et al.* 2010. The hexosamine biosynthetic pathway couples growth factor-induced glutamine uptake to glucose metabolism. *Genes Dev.* **24**: 2784–2799.

17. Ying H., A.C. Kimmelman, C.A. Lyssiotis, *et al.* 2012. Oncogenic Kras Maintains Pancreatic Tumors through Regulation of Anabolic Glucose Metabolism. *Cell* **149**: 656–670.

18. Guillaumond F., J. Leca, O. Olivares, *et al.* 2013. Strengthened glycolysis under hypoxia supports tumor symbiosis and hexosamine biosynthesis in pancreatic adenocarcinoma. *Proc. Natl. Acad. Sci. U. S. A.* **110**: 3919–3924.

19. Sharma N.S., V.K. Gupta, V.T. Garrido, *et al.* 2020. Targeting tumor-intrinsic hexosamine biosynthesis sensitizes pancreatic cancer to anti-PD1 therapy. *J. Clin. Invest.* **130**: 451–465.

20. Ricciardiello F., Y. Gang, R. Palorini, *et al.* 2020. Hexosamine pathway inhibition overcomes pancreatic cancer resistance to gemcitabine through unfolded protein response and EGFR-Akt pathway modulation. *Oncogene* **39**: 4103–4117.

21. Chen R., L.A. Lai, Y. Sullivan, *et al.* 2017. Disrupting glutamine metabolic pathways to sensitize gemcitabine-resistant pancreatic cancer. *Sci. Rep.* **7**: 7950.

22. Campbell S.L., C. Mesaros, H. Affronti, *et al.* 2020. Glutamine deprivation triggers NAGK-dependent hexosamine salvage. *bioRxiv* 2020.09.13.294116.

23. Kim P.K., C.J. Halbrook, S.A. Kerk, *et al.* 2020. Hyaluronic Acid Fuels Pancreatic Cancer Growth. *bioRxiv* 2020.09.14.293803.

24. Carey B.W., L.W.S. Finley, J.R. Cross, *et al.* 2015. Intracellular α-ketoglutarate maintains the pluripotency of embryonic stem cells. *Nature* **518**: 413–416.

25. Vardhana S.A., P.K. Arnold, B.P. Rosen, *et al.* 2019. Glutamine independence is a selectable feature of pluripotent stem cells. *Nat. Metab.* **1**: 676–687.

26. Ezhkova E., H.A. Pasolli, J.S. Parker, *et al.* 2009. Ezh2 orchestrates gene expression for the stepwise differentiation of tissue-specific stem cells. *Cell* **136**: 1122–1135.

27. Sen G.L., D.E. Webster, D.I. Barragan, *et al.* 2008. Control of differentiation in a self-renewing mammalian tissue by the histone demethylase JMJD3. *Genes Dev.* **22**: 1865–1870.

28. San Martín A., S. Ceballo, F. Baeza-Lehnert, *et al.* 2014. Imaging mitochondrial flux in single cells with a FRET sensor for pyruvate. *PloS One* **9**: e85780.

29. Peroza E.A., A.-H. Boumezbeur & N. Zamboni. 2015. Rapid, randomized development of genetically encoded FRET sensors for small molecules. *The Analyst* **140**: 4540–4548.

30. Cheng L.Y., A.P. Bailey, S.J. Leevers, *et al.* 2011. Anaplastic lymphoma kinase spares organ growth during nutrient restriction in Drosophila. *Cell* **146**: 435–447.

31. Bailey A.P., G. Koster, C. Guillermier, *et al.* 2015. Antioxidant Role for Lipid Droplets in a Stem Cell Niche of Drosophila. *Cell* **163**: 340–353.

32. Lubojemska A., M.I. Stefana, L. Lampe, *et al.* 2020. Adipose Triglyceride Lipase protects the endocytosis of renal cells on a high fat diet in Drosophila. *bioRxiv* 2020.11.19.390146.

33. Senyilmaz D., S. Virtue, X. Xu, *et al.* 2015. Regulation of mitochondrial morphology and function by stearoylation of TFR1. *Nature* **525**: 124–128.

34. Senyilmaz-Tiebe D., D.H. Pfaff, S. Virtue, *et al.* 2018. Dietary stearic acid regulates mitochondria in vivo in humans. *Nat. Commun.* **9**: 3129.

35. Hudry B., S. Khadayate & I. Miguel-Aliaga. 2016. The sexual identity of adult intestinal stem cells controls organ size and plasticity. *Nature* **530**: 344–348.

36. Reiff T., J. Jacobson, P. Cognigni, *et al.* 2015. Endocrine remodelling of the adult intestine sustains reproduction in Drosophila. *eLife* **4**: e06930.

37. Hudry B., E. de Goeij, A. Mineo, *et al.* 2019. Sex Differences in Intestinal Carbohydrate Metabolism Promote Food Intake and Sperm Maturation. *Cell* **178**: 901-918.e16.

38. Demontis F. & N. Perrimon. 2010. FOXO/4E-BP signaling in Drosophila muscles regulates organism-wide proteostasis during aging. *Cell* **143**: 813–825.

39. Rajan A. & N. Perrimon. 2012. Drosophila cytokine unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion. *Cell* **151**: 123–137.

40. Owusu-Ansah E., W. Song & N. Perrimon. 2013. Muscle mitohormesis promotes longevity via systemic repression of insulin signaling. *Cell* **155**: 699–712.

41. Demontis F., V.K. Patel, W.R. Swindell, *et al.* 2014. Intertissue control of the nucleolus via a myokine-dependent longevity pathway. *Cell Rep.* **7**: 1481–1494.

42. Song W., J.A. Veenstra & N. Perrimon. 2014. Control of lipid metabolism by tachykinin in Drosophila. *Cell Rep.* **9**: 40–47.

43. Kwon Y., W. Song, I.A. Droujinine, *et al.* 2015. Systemic organ wasting induced by localized expression of the secreted insulin/IGF antagonist ImpL2. *Dev. Cell* **33**: 36–46.

44. Song W., D. Cheng, S. Hong, *et al.* 2017. Midgut-Derived Activin Regulates Glucagon-like Action in the Fat Body and Glycemic Control. *Cell Metab.* **25**: 386–399.

45. Song W., E. Owusu-Ansah, Y. Hu, *et al.* 2017. Activin signaling mediates muscle-to-adipose communication in a mitochondria dysfunction-associated obesity model. *Proc. Natl. Acad. Sci. U. S. A.* **114**: 8596–8601.

46. Rajan A., B.E. Housden, F. Wirtz-Peitz, *et al.* 2017. A Mechanism Coupling Systemic Energy Sensing to Adipokine Secretion. *Dev. Cell* **43**: 83-98.e6.

47. Song W., S. Kir, S. Hong, *et al.* 2019. Tumor-Derived Ligands Trigger Tumor Growth and Host Wasting via Differential MEK Activation. *Dev. Cell* **48**: 277-286.e6.

48. Ghosh A.C., S.G. Tattikota, Y. Liu, *et al.* 2020. Drosophila PDGF/VEGF signaling from muscles to hepatocyte-like cells protects against obesity. *eLife* **9**:.

49. Bäckhed F., J.K. Manchester, C.F. Semenkovich, *et al.* 2007. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc. Natl. Acad. Sci. U. S. A.* **104**: 979–984.

50. Wang Y. & L.V. Hooper. 2019. Immune control of the microbiota prevents obesity. *Science* **365**: 316–317.

51. Wang Y., Z. Kuang, X. Yu, *et al.* 2017. The intestinal microbiota regulates body composition through NFIL3 and the circadian clock. *Science* **357**: 912–916.

52. Kuang Z., Y. Wang, Y. Li, *et al.* 2019. The intestinal microbiota programs diurnal rhythms in host metabolism through histone deacetylase 3. *Science* **365**: 1428–1434.

53. Yilmaz L.S. & A.J.M. Walhout. 2016. A Caenorhabditis elegans Genome-Scale Metabolic Network Model. *Cell Syst.* **2**: 297–311.

54. Yilmaz L.S., X. Li, S. Nanda, *et al.* 2020. Modeling tissue-relevant Caenorhabditis elegans metabolism at network, pathway, reaction, and metabolite levels. *Mol. Syst. Biol.* **16**: e9649.

55. Wei W., N.M. Riley, A.C. Yang, *et al.* 2021. Cell type-selective secretome profiling in vivo. *Nat. Chem. Biol.* **17**: 326–334.

56. Rappez L., M. Stadler, S. Triana, *et al.* 2019. Spatial single-cell profiling of intracellular metabolomes in situ. *bioRxiv* 510222.

57. Rabinovich S., L. Adler, K. Yizhak, *et al.* 2015. Diversion of aspartate in ASS1-deficient tumours fosters de novo pyrimidine synthesis. *Nature* **527**: 379–383.

58. Lee J.S., L. Adler, H. Karathia, *et al.* 2018. Urea Cycle Dysregulation Generates Clinically Relevant Genomic and Biochemical Signatures. *Cell* **174**: 1559-1570.e22.

59. Keshet R., P. Szlosarek, A. Carracedo, *et al.* 2018. Rewiring urea cycle metabolism in cancer to support anabolism. *Nat. Rev. Cancer* **18**: 634–645.

60. Mülleder M., F. Capuano, P. Pir, *et al.* 2012. A prototrophic deletion mutant collection for yeast metabolomics and systems biology. *Nat. Biotechnol.* **30**: 1176–1178.

61. Mülleder M., E. Calvani, M.T. Alam, *et al.* 2016. Functional Metabolomics Describes the Yeast Biosynthetic Regulome. *Cell* **167**: 553-565.e12.

62. Olin-Sandoval V., J.S.L. Yu, L. Miller-Fleming, *et al.* 2019. Lysine harvesting is an antioxidant strategy and triggers underground polyamine metabolism. *Nature* **572**: 249–253.

63. Campbell K., J. Vowinckel, M. Mülleder, *et al.* 2015. Self-establishing communities enable cooperative metabolite exchange in a eukaryote. *eLife* **4**:.

64. Zelezniak A., J. Vowinckel, F. Capuano, *et al.* 2018. Machine Learning Predicts the Yeast Metabolome from the Quantitative Proteome of Kinase Knockouts. *Cell Syst.* **7**: 269-283.e6.

65. Messner C.B., V. Demichev, D. Wendisch, *et al.* 2020. Ultra-High-Throughput Clinical Proteomics Reveals Classifiers of COVID-19 Infection. *Cell Syst.* **11**: 11-24.e4.

66. Demichev V., C.B. Messner, S.I. Vernardis, *et al.* 2020. DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. *Nat. Methods* **17**: 41–44.

67. Messner C.B., V. Demichev, N. Bloomfield, *et al.* 2021. Ultra-fast proteomics with Scanning SWATH. *Nat. Biotechnol.*

68. Fan J., J. Ye, J.J. Kamphorst, *et al.* 2014. Quantitative flux analysis reveals folate-dependent NADPH production. *Nature* **510**: 298–302.

69. Brown S., C.M. Pineda, T. Xin, *et al.* 2017. Correction of aberrant growth preserves tissue homeostasis. *Nature* **548**: 334–337.

70. Pineda C.M., D.G. Gonzalez, C. Matte-Martone, *et al.* 2019. Hair follicle regeneration suppresses Ras-driven oncogenic growth. *J. Cell Biol.* **218**: 3212–3222.

71. Horak M., J. Novak & J. Bienertova-Vasku. 2016. Muscle-specific microRNAs in skeletal muscle development. *Dev. Biol.* **410**: 1–13.

72. Wang J., L.Z. Yang, J.S. Zhang, *et al.* 2018. Effects of microRNAs on skeletal muscle development. *Gene* **668**: 107–113.

73. Ni M., A. Solmonson, C. Pan, *et al.* 2019. Functional Assessment of Lipoyltransferase-1 Deficiency in Cells, Mice, and Humans. *Cell Rep.* **27**: 1376-1386.e6.

74. Pirot N., M. Crahes, H. Adle-Biassette, *et al.* 2016. Phenotypic and Neuropathological Characterization of Fetal Pyruvate Dehydrogenase Deficiency. *J. Neuropathol. Exp. Neurol.* **75**: 227–238.

75. Chakrabarty R.P. & N.S. Chandel. 2021. Mitochondria as Signaling Organelles Control Mammalian Stem Cell Fate. *Cell Stem Cell* **28**: 394–408.

76. Ansó E., S.E. Weinberg, L.P. Diebold, *et al.* 2017. The mitochondrial respiratory chain is essential for haematopoietic stem cell function. *Nat. Cell Biol.* **19**: 614–625.

77. Bricker D.K., E.B. Taylor, J.C. Schell, *et al.* 2012. A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, Drosophila, and humans. *Science* **337**: 96–100.

78. Schell J.C., K.A. Olson, L. Jiang, *et al.* 2014. A role for the mitochondrial pyruvate carrier as a repressor of the Warburg effect and colon cancer cell growth. *Mol. Cell* **56**: 400–413.

79. Schell J.C., D.R. Wisidagama, C. Bensard, *et al.* 2017. Control of intestinal stem cell function and proliferation by mitochondrial pyruvate metabolism. *Nat. Cell Biol.* **19**: 1027–1036.

80. Cluntun A.A., R. Badolia, S. Lettlova, *et al.* 2021. The pyruvate-lactate axis modulates cardiac hypertrophy and heart failure. *Cell Metab.* **33**: 629-648.e10.

81. Bensard C.L., D.R. Wisidagama, K.A. Olson, *et al.* 2020. Regulation of Tumor Initiation by the Mitochondrial Pyruvate Carrier. *Cell Metab.* **31**: 284-300.e7.

82. Orsak T., T.L. Smith, D. Eckert, *et al.* 2012. Revealing the allosterome: systematic identification of metabolite-protein interactions. *Biochemistry* **51**: 225–232.

83. Dai Z., V. Ramesh & J.W. Locasale. 2020. The evolving metabolic landscape of chromatin biology and epigenetics. *Nat. Rev. Genet.* **21**: 737–753.

84. Reid M.A., Z. Dai & J.W. Locasale. 2017. The impact of cellular metabolism on chromatin dynamics and epigenetics. *Nat. Cell Biol.* **19**: 1298–1306.

85. Mentch S.J., M. Mehrmohamadi, L. Huang, *et al.* 2015. Histone Methylation Dynamics and Gene Regulation Occur through the Sensing of One-Carbon Metabolism. *Cell Metab.* **22**: 861–873.

86. Orentreich N., J.R. Matias, A. DeFelice, *et al.* 1993. Low methionine ingestion by rats extends life span. *J. Nutr.* **123**: 269–274.

87. Gao X., S.M. Sanderson, Z. Dai, *et al.* 2019. Dietary methionine influences therapy in mouse cancer models and alters human metabolism. *Nature* **572**: 397–401.

88. Ahlqvist K.J., R.H. Hämäläinen, S. Yatsuga, *et al.* 2012. Somatic progenitor cell vulnerability to mitochondrial DNA mutagenesis underlies progeroid phenotypes in Polg mutator mice. *Cell Metab.* **15**: 100–109.

89. Ahlqvist K.J., A. Suomalainen & R.H. Hämäläinen. 2015. Stem cells, mitochondria and aging. *Biochim. Biophys. Acta* **1847**: 1380–1386.

90. Trifunovic A., A. Wredenberg, M. Falkenberg, *et al.* 2004. Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* **429**: 417–423.

91. Kujoth G.C., A. Hiona, T.D. Pugh, *et al.* 2005. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science* **309**: 481–484.

92. Hämäläinen R.H., J.C. Landoni, K.J. Ahlqvist, *et al.* 2019. Defects in mtDNA replication challenge nuclear genome stability through nucleotide depletion and provide a unifying mechanism for mouse progerias. *Nat. Metab.* **1**: 958–965.