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Metabolic decisions in development and disease—a Keystone Symposia report

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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.

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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. 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 discussions explored how metabolites impact cellular and developmental decisions in a diverse range of model systems used to investigate normal development, developmental disorders, dietary effects, and cancer-mediated changes in metabolism.

Keywords: cell signaling; development; inborn errors of metabolism; metabolism; metabolome; stem cell differentiation

Introduction

Once regarded as a housekeeping process, metabolism is increasingly being appreciated as a driver for cell signaling and cell decision making. Several lines of evidence indicate that metabolites are not just passive building blocks for generating cellular biomass and energy; for example, some metabolites can serve as substrates for protein and DNA modifications. Protein glycosylation depends on the 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 also often associated with changes in cell state.

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 to investigate normal development, developmental disorders, dietary effects, and cancer-mediated changes in metabolism.

Several symposia speakers discussed how metabolites can serve as active signaling molecules, providing a link between metabolic pathways and classic cell signaling pathways, while others discussed how metabolites can influence cell fate and differentiation. For example, the metabolite α-ketoglutarate can promote epidermal stem cell differentiation by promoting DNA demethylation.[3] In addition, H_2O_2 generated by metabolic pathways in 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 a 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.

Another group of speakers presented new techniques to investigate the metabolome. Studying the metabolome requires sophisticated computational techniques because of the large number of metabolites, the fact that they can be generated via multiple pathways, and the complicated feedback loops that affect their production. Some 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.

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. 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 during which neuromesodermal precursors (NMPs) 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 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 reach of FGF and WNT signaling, they are sensitive to the segmentation clock and, as a response, activate genes involved in segmentation.

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, which 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]

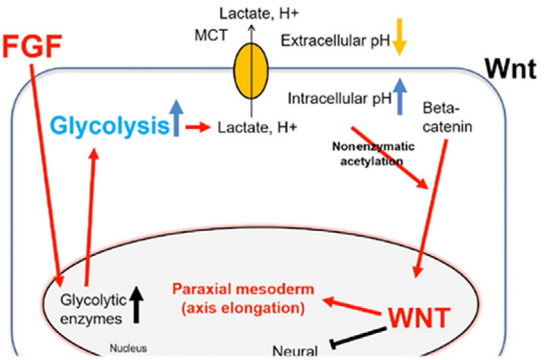

Figure 1. **Model for how glycolytic regulation of intracellular pH and WNT signaling controls cell differentiation in NMPs in the presomitic mesoderm.**

Work from Pourquié's lab 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 in which the intracellular pH is higher than the extracellular pH.[9] Similarly, the tail bud has an inverted pH gradient in which the posterior region has a more acidic extracellular pH and more 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 nonenzymatic acetylation of β-catenin, which promotes mesoderm induction.[11] Pourquié showed that β-catenin acetylation is dependent on glycolysis both *in vitro* and *in vivo*.[12]

Pourquié proposed a model (Fig. 1) showing that 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

nonenzymatic acetylation of β-catenin, which promotes WNT signaling and leads to the 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 the production of glycosyl donors like UDP-GlcNAc, while acetyl-CoA is the acetyl donor for acetylation.

Kathryn Wellen, from the University of Pennsylvania, described how the cell can achieve specificity in metabolic regulation of the epigenome. Wellen asserted that the nucleus should be considered at least in part a distinct metabolic compartment and that spatiotemporal 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, in close collaboration with the lab of Nathaniel Snyder, has developed an approach to subcellular acyl-CoA quantification dubbed stable isotope labeling of essential nutrients in cell culture with subcellular 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. 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, 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, leveraging elevated GlcNAc pools, potentially released from 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), which phosphorylates GlcNAc. 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 as the tumor environment is more nutrient restricted.[22]

Wellen proposed that under high-nutrient conditions, 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 cosubstrates 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, from Memorial 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 are sufficient to alter cell fate. Finley argued that metabolism should be considered coequal to cell signaling in regulating cell identify. Finley described work in two types of mouse embryonic stem cells (ESCs): naive ESCs, which represent the earliest pluripotency state that can be captured *in vitro*, and metastable ECSs, which have a more committed phenotype.

Finley showed that naive cells shuttle more glucose-derived carbons and less glutamine-derived carbons into the TCA cycle than metastable cells. This metabolic difference has functional consequences: similar to most cells, metastable ESCs do not proliferate under glutamine deprivation, while naive ESCs do.[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 because the most committed cells died.[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 normally produced using the carbon backbone of α-ketoglutarate; in naive cells, high levels of α-ketoglutarate correlate with 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 α-ketoglutarate can also tune demethylation in adult epidermal stem cells and thus promote differentiation. Depriving epidermal stem cells of serine, a critical donor for methylation, reduced histone methylation and induced differentiation. Finley showed that production of α-ketoglutarate via the serine synthesis pathway drives, not lack of serine itself, demethylation and cell differentiation. In an allograft model, tumors were sensitive to serine starvation, demonstrating how targeting metabolic pathways to control cell fate might lead to anticancer strategies.[3]

Together, the results finley presented support a model by which α-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 a PYRATE (PYRuvATE Sensor)/FRET ratio gradient was linked to presomitic mesoderm differentiation.[8] The presomitic mesoderm contains a signaling gradient (see pg. 3), 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 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 Institute, presented work on how developing animals cope with and respond to stresses in their environments. To answer these questions, Gould's group is developing *Drosophila* models to investigate different types of development stress, such as nutrient restriction and hypoxia. Gould focused on comparing the roles of lipid metabolism in the developing central nervous system (CNS) and renal system.

Gould's lab has shown that glia are part of the neural stem cell niche and play important roles in initiating and maintaining neuroblast proliferation in the *Drosophila* larval CNS.[30,31] During hypoxia, neuroblast

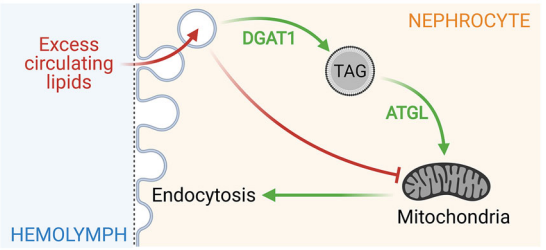

Figure 2. **Excess circulating lipids are endocytosed by nephrocytes and trafficked to lipid droplets and then to mitochondria. However, when the system is overwhelmed, endocytosed lipids take a nonlipid droplet route that is toxic to mitochondria.**

divisions are protected by lipid droplets produced in the glial niche.[30,32] Glial-specific knockdown of the enzyme DGAT1, which synthesizes triacylglycerol, a key component of these lipid droplets, increased lipid peroxidation and decreased neural stem cell proliferation.[32] This suggests that the biosynthesis of glial lipid droplets plays a beneficial role in the developing CNS. Given that other studies of lipid droplets in different contexts have reported opposite effects, Gould emphasized the need for additional systematic comparisons.

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, an 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 circulating macromolecules. Nephrocyte overexpression of ATGL, a lipase localized to lipid droplets that hydrolyses triacylglycerols to release fatty acids, rescued these phenotypes. Gould put forth a model (Fig. 2) wherein flux of fatty acids 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, if the system is overwhelmed with lipids, such as in the case with an HFD, then the lipids go through a yet-to-be determined nonlipid droplet route, which is toxic to nephrocyte mitochondria.[33]

Broadly speaking, Gould's work shows that stress-induced lipid droplets play similar roles in two very different developmental contexts, the neural stem cell niche and the renal system. In both cases, fatty acid flux through triacylglycerol-containing lipid droplets appears to minimize lipid peroxidation and to protect 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; in many cases, however, the concentration of the metabolite itself can be rate limiting.

Aurelio Teleman, from the German Cancer Research Center (DKFZ), 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 is found in the diet and can also be formed from palmitic acid via the enzyme ELOVL6. Teleman's team showed that in flies, *Elovl6* knock out 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 have defective mitochondria that are 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 the addition of stearic acid.[34]

The Teleman group 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.[34]

This mechanism is biologically relevant both in flies and humans. Flies fed a diet lacking stearic acid had speckled/unfused mitochondria, which resolved with a stearic acid–containing diet.[34] 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 3 h of eating a high

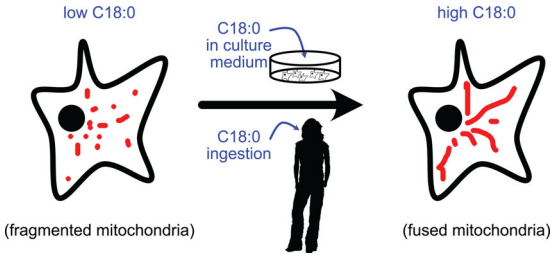

Figure 3. **Metabolites present in food, like stearic acid, can have direct effects on cell signaling via post-translational modifications.**

stearic acid meal, the proportion of fragmented mitochondria decreased, while the proportion of fused mitochondria increased.[35]

Teleman's group is using mass spectrometry to identify other proteins modified by stearic acid.[36] In addition to the transferrin receptor, GNAI proteins are also modified when cells are exposed to 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.[36]

This work (Fig. 3) shows that metabolites present in food, like stearic acid, can have direct effects on cell signaling via post-translational modifications. In addition, it highlights the need to shift the current thinking of lipid modification from a binary on/off switch to a view that incorporates the differential effects of specific modifications.

Short talk: tryptophan metabolism in cell fate

William Tu, from Kathrin Plath's lab at the David Geffen School of Medicine at UCLA, presented 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 into the genomic and molecular features of this process, less is known about the role that metabolism may play. Tu is investigating whether dietary nutrients can regulate cell fate transition and identifying the mechanisms behind metabolic control of cell fate transition. Tu found that the addition of the essential amino acid tryptophan enhanced the reprogramming of differentiated cells to iPS cells. This effect was mediated through downstream metabolites of the tryptophan pathway and tryptophan-dependent gene expression programs. This work shows how specific diet-derived metabolites 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

Diet–microbiome interactions in CKDs and cancer

Wendy Sarah Garrett, from the Harvard T. H. Chan School of Public Health and Dana-Farber Cancer Institute, presented work on how dietary sulfur amino acids can modulate microbiome distinct physiological activities, with relevance for health kidney function and tumor immunity. While much of dietary–microbiome research has focused on how dietary shifts modulate the composition of the gut microbiome, Garrett's lab has found that diet can affect the activity of distinct microbial enzymes rather than composition of the gut microbiome. Garrett pointed out that while sulfur amino acids and the gasotransmitter H_2S have been studied extensively in mammalian systems, they have been underinvestigated in relation to the gut bacterial proteome. Among the points Garrett emphasized was how to exploit the power of gnotobiotics to perform biochemical mapping of gut microbiome enzymatic activity and metabolic outputs with a focus of model CKDs. Upon defining how the sulfur amino acid cysteine can shape the S-sulfhydrome of *E. coli*, Garrett's lab was intrigued to find the highly conserved bacterial enzyme tryptophanase as a top hit among several S-sulfhydrated proteins. Tryptophanase catalyzes the conversion of tryptophan to indole and indole as pleitropic effects in bacterial–bacterial communication, host immunity, and kidney function. Indole and its metabolite indoxyl sulfate are uremic toxins—nephrotoxic molecules that build up in patients with CKD as they are renally cleared.

Using both conventional and gnotobiotic mouse models of healthy mice and mice with kidney disease, Garrett's lab found that modulating the levels of cysteine and methionine (above levels in amino acid or dietary restriction) caused marked

effects on kidney function in healthy mice and could also affect the progression of kidney disease.[37] High dietary levels (but not so high to cause health issues in mice or humans) kept kidney function at a normal level in healthy mice and ameliorated disease in a kidney disease model; low dietary levels of sulfur amino acids negatively impacted kidney function in healthy mice and exacerbated the severity of renal failure in the disease models. A key (but by no means sole) driver of the positive effects of the high sulfer amino acid diet pointed to tryptophanase and the diet via bacterial conversion of cysteine to H_2S, resulting in post-translation modification of tryptophanase's active site cysteines. The dietary shift from "low" to "high" sulfur amino acids changed the S-sulfydration patterns of bacterial tryptophanase, which in turn affected the levels of indole and indoxyl sulfate in the mice. Using designer microbial communities with different gene knockout bacterial strains in gnotobiotic mice, the Garrett's lab was able to demonstrate the biological consequence of dietary-mediate S-sulfydration for gut microbial enzymatic activity and mouse kidney function. The lab is looking to bring such simple dietary measures to help the over 850 million people worldwide with CKDs.

In the second part of her talk, Garrett discussed roles for diet–microbe interactions in enhancing antitumor immunity. Using a similar diet to the one studied by her group in CKDs, they found that in some mice dietary sulfur amino acids (in the diet) could change the thickness of intestinal mucus, creating a niche for bacterial taxa that modulate the immune system. Many cancers, for example colon cancer, are not very responsive to immunotherapy; Garrett discussed how analyses of gut microbiome datasets from patients responsive to immunotherapies can be leveraged to identify bacteria that enhance antitumor immunity for patients with tumors not traditional responsive to immunotherapies.

Interorgan metabolic crosstalk in Drosophila

Irene Miguel-Aliaga, from Imperial College London, presented work on understanding the metabolic crosstalk among organs in *Drosophila*, specifically between the gut and other organs. Gut neurons, hormones, microbes, immune cells, and metabolites communicate with non-gut 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 (ISCs), epithelial cells, and enterocytes. Prior work in Miguel-Aliaga's lab showed that the guts of male and female flies show differences at multiple levels, including gene expression and physiological features. Initially, they 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.[38,39]

Miguel-Aliaga's group has been systematically characterizing sex differences in different cell types of the fly gut, for example, 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.[40]

Miguel-Aliaga's group found that ISC 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.[38,40]

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 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.[40]

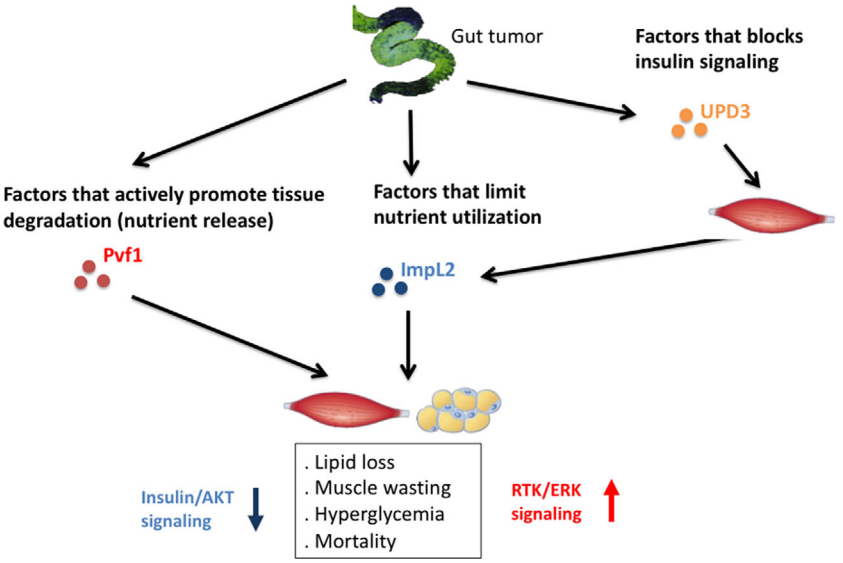

Figure 4. Model for organ wasting via crosstalk between tumors and peripheral organs.

Miguel-Aliaga put forth a model in which the testes secret testis cytokine to increase glucose metabolism in enterocytes, which in turn increases food consumption and citrate secretion that 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, from Harvard Medical School, presented 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.[41–51] Perrimon focused on organ wasting in cancer models in fruit flies.[46] 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 by the muscle, leading to lipid loss, muscle wasting, and hyperglycemia.[46] 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 (Fig. 4).

Perrimon described work to systematically identify inter-organ communication factors and analyze their systemwide effects using snRNA-seq and proximity labeling. He 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, from The University of Texas Southwestern Medical Center, presented 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 mechanisms. The microbiota can break down dietary components such as

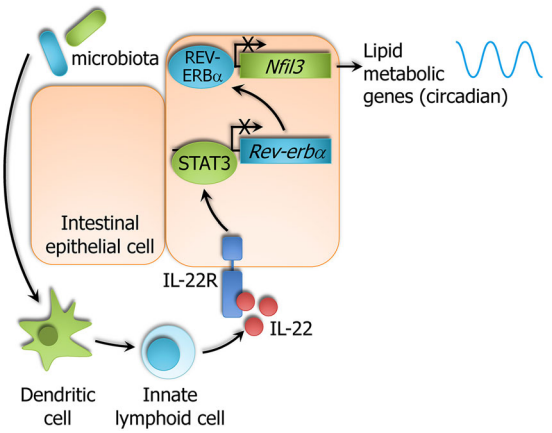

Figure 5. **Description of how the microbiota influence *Nfil3* expression via interactions with subepithelial immune cells.**

polysaccharides into simple sugars that are more easily absorbed. Hooper's described how these interactions can be more complicated. Their work has demonstrated 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 (Fig. 5).

It has been known that the gut microbiota can impact fat storage and body composition. Germ-free mice have a lower body fat percentage than conventionally raised mice and are protected from the effects of a high-fat diet (HFD) on weight and body composition.[52] 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* knockout mice showed that NFIL3 regulates a circadian metabolic gene transcription program centered on fatty acid metabolism. Hooper showed that microbiota triggers circadian expression of epithelial NFIL3, which regulates the 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 subepithelial immune cells stimulate NFIL3 expression via IL-22R. Inside the epithelial cell, activation of IL-22R activates STAT3, which downregulates REV-ERBα, a component of the circadian clock and a repressor of NFIL3 expression.[53,54]

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.[55] Classically, HDAC3 is a histone deacetylase that regulates chromatin accessibility. Hooper showed that it can have other roles as well. The microbiota can affect HDAC3 expression. In germ-free mice, HDAC3 expression is lower than in mice that have gut microbiota. Similar to the *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. Hooper's lab is trying to tease out this complexity by determining the effects of specific gut bacteria on lipid metabolism pathways.

Toward genome-scale personalized metabolic networks

A. J. Marian Walhout, from the University of Massachusetts Medical School, presented work on developing personalized metabolic networks in *Caenorhabditis elegans*. Walhout's lab is broadly interested in the effects of nutrients on gene expression and physiology, and in how metabolism and gene expression interact at a system level. The goal is to integrate nutrigenetics, genomics, and transcriptomics to understand how diet affects individuals, how to predict health outcomes, and how to 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.[56]

The model was recently updated to include more genes and scRNA-seq data to predict tissue-relevant metabolism at the network, pathway, reaction, and metabolite levels[57] (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 at Stanford University, presented 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[58,59] to the ER–Golgi, while a cytosolic TurboID is used to map unconventional secretion. A different labeling enzyme, subtiligase,[60,61] that ligates exogenous biotinylated peptide ester to neo-termini of cleaved polypeptides, 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 betaine-homocysteine S-methyltransferase (BHMT), an enzyme involved in methionine metabolism that had not previously been known to be secreted.[62] 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, from EMBL and UCSD, presented work on spatial and single-cell metabolomics. Alexandrov's lab has developed a method, SpaceM, that provides single-cell metabolomics information.[63] In brief, cells grown in culture are fixed on slides and imaged by using light microscopy. This provides information on cell image, morphology, fluorescent readouts, 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. The imaging mass spectrometry setup used by Alexandrov allows the pixel size to be 5 μm, and spectra contain information on over 100 molecules, including metabolites, lipids, and drugs. This method generates a wealth of single-cell data, including mass spectra intensities of hundreds of metabolites, lipids, and 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.[63] Alexandrov presented recently published data showing how SpaceM can be used to reveal coexisting metabolic states of steatotic hepatocytes 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 crosstalk between 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. The complete urea cycle occurs in both the mitochondria and cytosol of hepatocytes, and most healthy tissues express some urea cycle enzymes. In cancer cells, urea cycle enzymes are dysregulated to increase the availability of nitrogen-rich compounds for synthesis of pyrimidines and amino acids. These excess nutrients support

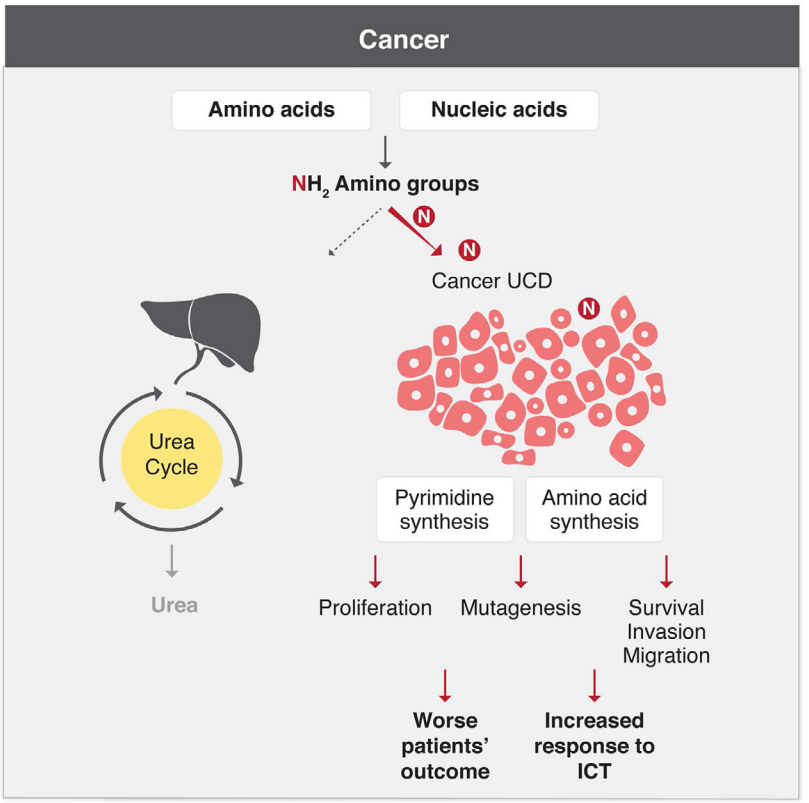

Figure 6. **The urea cycle is upregulated in tumor cells, promoting carcinogenesis.**

cancer growth and promote mutagenesis, ultimately leading to poorer outcomes.[64–66]

Erez also described work showing changes in the urea cycle in the liver (Fig. 6). 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 with matched controls, suggesting that urea cycle dysregulation may be a common feature of cancer.[65]

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 Institute and the Charité – Universitätsmedizin Berlin, presented research on understanding the connectivity from genome, to proteome, to metabolome. Ralser's talk focused on using metabolic perturbations to derive the logic of metabolism.

When cells are grown in the presence of nutrient supplementations, cells effectively switch off many of their own biosynthetic pathways and use the extracellular metabolites instead. This situation makes it difficult to study metabolism and is partly why many genes are still without functional annotation (i.e., they are not needed under laboratory growth conditions). Ralser's group has created thousands of metabolically competent yeast strains in which many of the metabolic genes have been restored. Using these strains, they have developed high-throughput methods for cell cultivation, metabolite extraction, and proteomics.[67,68] One of the first applications of this resource 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 linked each yeast gene to a metabolic phenotype.[69] This work also revealed some novel roles for amino acids. For example, Ralser showed that yeast cells can actively take up lysine to concentrations much higher than required for growth. "Lysine harvesting" reconfigures metabolism and makes the cells more tolerant to oxidative stress.[70]

Amino acid metabolism occurs not only within cells but also between cells. Yeast can form self-establishing communities in which cells share metabolites.[71] 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.[72] They have recently modified their proteomics platform to scale up from hundreds to thousands of proteomes.[68,73,74]

Elucidating tissue-specific lipogenesis pathways

Joshua Rabinowitz, from Princeton University, presented work on delineating the mechanisms of *de novo* lipogenesis in the liver and in adipose tissue. *De novo* lipogenesis is a hallmark of nonalcoholic 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 and 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 activities of malic enzyme and isocitrate dehydrogenase 1 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.[75]

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 in particular those involved in pathology such as liver *de novo* lipogenesis in fatty liver disease, can reveal actionable targets for therapeutics.

Short talk: visualizing metabolic outcomes of oncogenic mutations in the skin

Anupama Hemalatha, a postdoctoral researcher from Valentina Greco's lab at Yale University, presented work on understanding metabolic changes induced by oncogenic mutations by *in vivo* imaging of redox state while a tissue is adapting to 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 β-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.[76,77]

Hemalatha presented unpublished work that combines live mouse skin imaging with optical redox ratio imaging (developed in Melissa Skala's lab)[78] to monitor how these oncogenic mutant cells affect metabolic activity in the stem cell layer of the epidermis, from their induction, to elimination out of, or integration into, the homeostatic tissue.

Short talk: miR-1 sustains muscle physiology by controlling V-ATPase complex assembly

Paula Gutiérrez-Pérez, from Luisa Cochella's lab at the Research Institute of Molecular Pathology, presented work on understanding how miR-1 supports muscle physiology. miR-1 is a deeply conserved, muscle-specific microRNA whose depletion has been linked to several defects in cardiac and skeletal muscle development and function.[79,80] While several putative downstream targets of miR-1 have been proposed, none are conserved. Gutiérrez-Pérez showed that the main and conserved role of miR-1 is to control several subunits of the V-ATPase complex, which is ultimately essential to guarantee the assembly of this intricate complex in muscle cells. These results reveal a novel role for this microRNA in regulating protein complex assembly, and highlight the importance of mitochondrial function and proteostasis in muscle physiology.[81]

Metabolic disorders

Investigation inborn errors of metabolism

Ralph DeBerardinis, from the University of Texas Southwestern Medical Center, presented work on

how genetic mutations result in disease through their effects on metabolism. DeBerardinis 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 University of Texas 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 characterized pattern in the patient's metabolomic profile that consisted of high levels of lactate, proline, alanine, and glutamate. 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.[82] DeBerardinis' 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 on 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.[83] While it is unclear why these defects occur, this demonstrates that specific pathways are important for different aspects of development. DeBerardinis' group has developed a system to assess metabolic properties during gestation in developing embryos and placenta in mice. DeBerardinis 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, from Northwestern University, presented work on understanding the mitochondron as a signaling organellon. 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 (Fig. 7).

Mitochondria generate a variety of signals that can control stem cell fate and function, including H_2O_2, NAD/NADH, fumarate, and succinate. Mitochondrial H_2O_2 is necessary for adipocyte differentiation from human mesenchymal stem cells,[4] as well as for 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 H_2O_2 to drive normal differentiation. Overproduction of H_2O_2 can lead to stem cell exhaustion and depletion, while underproduction or exposure to antioxidants can cause stem cells to undergo cell death.[84]

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, as well as increased DNA and histone methylation, which ultimately inhibited differentiation.[85]

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 mitochondria.[86] 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.[87–90]

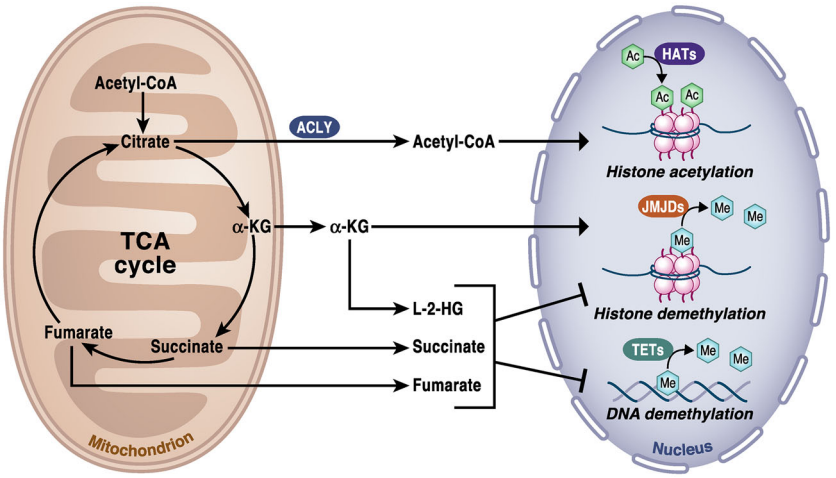

Figure 7. **Mechanisms by which mitochondrial metabolism can affect DNA and histone modifications and subsequently gene expression and cell differentiation.**

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, proteins are restricted to one side of the chamber. Metabolites in the two chambers are quantified by mass spectrometry. Those that bind to proteins will be enriched in the protein-containing chamber.[91]

Rutter showed unpublished work using MIDAS to characterize protein–metabolite interactions for hundreds of proteins involved in metabolic pathways or growth factor signaling. Rutter's 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 computation 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, e.g., 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.[92] 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.[93]

Locasale focused on methionine metabolism, which processes the carbon unit for methylation. Methionine concentration in the plasma is highly variable,[94] and most of this 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 life span and body weight.[95] Locasale showed that methionine concentrations can range three- to four-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.[96] They also showed that methionine metabolism can influence histone methylation, thus linking methionine to chromatin dynamics and potentially changes in cellular state.[94]

Short talk: mitochondrial defects and premature aging

Juan C. Landoni, from Anu Suomalainen'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 in somatic progenitor and stem cell pools. However, one progeric mouse model, the mitochondrial DNA (mtDNA) mutator mouse, contains defects in the DNA 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 and team characterized stem cells from the mtDNA mutator mouse. They showed that mtDNA mutator iPSCs and somatic precursors exhibit delays in cell proliferation and cell cycle progression, as well as nuclear DNA damage (Fig. 8). They showed that defects in PolG increase the replication rate of mtDNA, causing cells to preferentially distribute dNTPs to mitochondria over the nucleus. This leads to dNTP deficiency in the nucleus, replication stalling, and DNA damage in stem/progenitor cells. Landoni's work shows that instead of providing support for the mitochondrial theory of aging, the mtDNA mutator mouse model demonstrates accelerated aging via similar mechanisms as other models, namely, via nuclear genome instability.[92]

Metabolic regulation of cell fate decisions

Heather Christofk, from the David Geffen School of Medicine at UCLA, presented work on how nutrients can affect stem cell fate decisions. Before

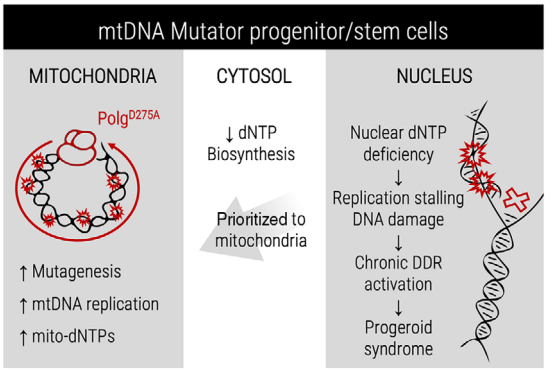

Figure 8. Models of accelerated aging that target mtDNA replication also result in increased nuclear genome instability, similar to other models of accelerated aging.

the evolution of multicellular organisms, cells relied on heavily nutrient cues from the environment to control their possible fates, such as whether to migrate, grow, or divide. Most primitive aspects of signaling that relay nutrient information to cell fate decisions are likely preserved in mammalian cells, although systemically and locally released signaling molecules, such as hormones, growth factors, and cytokines, can also regulate cell fate and function.[97]

The Christofk lab, in collaboration with several stem cell and developmental biology labs at UCLA, has screened nutrients that vary in the blood owing to diet for ability to impact cell fate decisions in multiple differentiation systems. Christofk showed unpublished results from a nutrient screen in cholangiocyte-derived hepatic organoids, a model of the oval cell response and liver regeneration from toxic liver injury.[98] They found that ascorbic acid (vitamin C) dose-dependently reduces hepatic organoid growth and progenitor marker expression, likely through impacting the activity of an alpha ketoglutarate-dependent dioxygenase enzyme, which uses ascorbic acid as an enzymatic cofactor.[99] The Christofk lab is now narrowing down which alpha-ketoglutarate–dependent dioxgenase enzyme is responsible for the ascorbic acid–mediated effects on hepatic organoids and is testing how ascorbic acid levels in the diet impact recovery from toxic liver injury in *Gulo*$^{-/-}$ mice (mice, like humans, obtain all of their ascorbic acid from their diet).

Christofk also discussed the need for better methods and tools to study *in vivo* tissue stem cell metabolism and draw causal relationships between

metabolism and cell fate change.[100] She showed one new approach of using antibody-conjugated magnetic beads for rapid isolation of hair follicle stem cells for metabolomic characterization, eliminating the long times and mechanical stress imposed by cell sorting.

Last, Christofk discussed how her group is mapping metabolism in developing fetuses in collaboration with the Nakano lab at UCLA. They are infusing pregnant mice with stable isotope–labeled nutrient tracers and then dissecting fetal hearts, livers, brains, as well as placentas at different stages of mid-gestation, e.g., E10, E12, E15, and E18. They are also assessing how maternal hyperglycemia affects fetal metabolism and have found an increase in nucleotide levels in developing hearts in the fetuses isolated from Akita mice, a murine model of diabetic pregnancy. These findings are consistent with previous results from the Nakano and Christofk labs showing that high glucose levels impair cardiomyocyte maturation from hESCs through elevated nucleotide biosynthesis.[101]

Competing interests

J.W.L. is a paid advisor to Restoration Foodworks.

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