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MEETING REVIEW

Metabolic decisions in development and disease

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ABSTRACT

The intimate relationships between cell fate and metabolism have long been recognized, but a mechanistic understanding of how metabolic pathways are dynamically regulated during development and disease, how they interact with signalling pathways, and how they affect differential gene expression is only emerging now. We summarize the key findings and the major themes that emerged from the virtual Keystone Symposium 'Metabolic Decisions in Development and Disease' held in March 2021.

KEY WORDS: Metabolism, Cell fate, Metabolic plasticity, Nutrition, Development

Introduction

Metabolism and biochemistry have long been studied *in vitro* or in homogenized cells and tissues. This has led to an in-depth knowledge of core metabolic pathways. However, we are only now beginning to understand the diverse roles and dynamic regulation of these metabolic pathways in the context of specific cells, tissues, organisms and stages of life. The observation of Otto Warburg (Warburg, 1956), that aerobic glycolysis was a hallmark of cancer cells, has long been the go-to example of how central carbon metabolism can be rewired to meet the biosynthetic needs of proliferation. But a lack of sensitive and tailored tools has prevented researchers from moving beyond correlation and from distinguishing cause from consequence. In this virtual Keystone Symposia on 'Metabolic Decisions in Development and Disease', organized by Irene Miguel-Aliaga, Ralph DeBerardinis and Marian Walhout, it became clear that the field of developmental and disease metabolism is maturing and moving on. Over the course of these 2 days, the work of Warburg, and the important influence it long had on this budding field, was mentioned only a few times. Instead, Warburg's contemporaries were cited, and the many different physiological and pathological aspects of metabolism they explored (e.g. Spratt, 1950). Building on these early correlative studies, we were nurtured with a wide range of energizing stories, ranging from bioenergetic pathways that adopt signalling functions, over metabolic compartmentalization, metabolic plasticity and metabolic cooperation between different organelles, organs and organisms, to the impact of nutrition on development and disease. All of this was topped with exciting new technologies to feed the next steps in this dynamic and highly interdisciplinary field.

Metabolic regulation of cell fate decisions

After early studies into metabolic requirements of development and disease in the 19th and early 20th century (Spratt, 1950; Warburg, 1956), the golden era of genetics and molecular biology that followed meant metabolism had to make room for signalling pathways and transcription factors as the main players in developmental and tumour biology. More recently, it has become evident that metabolic pathways are not only there to support cell-type- and context-specific bio-energetic demands, but also to play instructive roles with clear metabolic signalling functions that ultimately determine normal and pathological cell fate decisions. The keynote talk by Olivier Pourquié (Harvard Medical School, Boston, MA, USA) gave an excellent example of how the field is moving from correlative to more functional studies. Working in the posterior presomitic mesoderm, a classic model for developmental patterning and morphogenesis, his lab found spatial differences in expression of glycolytic genes that control tail bud elongation (Oginuma et al., 2017). Similar findings were reported by Alexander Aulehla (EMBL, Heidelberg, Germany) (Bulusu et al., 2017), and both speakers gave fascinating accounts of their work to dissect the interactions between glycolysis and developmental signalling pathways.

The Pourquié lab identified complementary extra- and intracellular pH gradients along the elongating body axis. Taking advantage of an *in vitro* system of pluripotent stem cell-derived presomitic mesoderm differentiation (Diaz-Cuadros et al., 2020), they found that manipulation of glycolysis and the pH affect Wnt activity. Intriguingly, non-enzymatic acetylation of β-catenin is dependent on intracellular pH and promotes mesodermal, rather than neuro-ectodermal, differentiation, providing a possible mechanism through which glycolysis-dependent pH controls a Wnt-mediated developmental switch (Oginuma et al., 2020). It will be interesting to see how widespread this non-enzymatic modification of proteins is and whether, for example, it may also apply to the tightly regulated acetylation of histones. Axis elongation is linked to periodic somite formation. Aulehla's group developed a transgenic mouse model to increase glycolytic activity by PFKFB3 overexpression (Yalcin et al., 2009), resulting in Wnt signalling downregulation and also in a slowing of segmentation clock oscillations. They next showed that the segmentation clock can be entrained not only by periodic Notch inhibition, but also by manipulating glucose concentration. This demonstrates the power of tailored functional perturbations, even in a living embryo, thanks to the development of advanced genetic tools. Combining these with an expanding range of genetically encoded metabolite sensors will allow for more mechanistic *in vivo* studies on the instructive roles played by key metabolic pathways and states such as glycolysis and the pH.

Further demonstrating the power of mouse genetics, Navdeep Chandel (Northwestern University, Evanston, IL, USA) sketched how, over the past three decades, our appreciation of mitochondria has evolved from simple 'potato-shaped organelles' that support bioenergetics and biosynthesis to highly dynamic signalling organelles that drive key cell fate decisions. The best-established mitochondrial-derived signalling molecules are reactive oxygen species (ROS), which, for example, act downstream of the

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Notch-signalling pathway to mediate keratinocyte differentiation (Hamanaka et al., 2013). Chandel' s most recent work focused on the role of mitochondrial complex I in regulating differentiation of lung alveoli through its role in maintaining redox balance and α-ketoglutarate concentration.

Many other nutrients and metabolites were discussed for their instructive roles in determining cell fate decisions, which they often do by directly or indirectly affecting epigenetic modifications and gene expression. William Tu (from the lab of Kathrin Plath, UCLA, USA) showed how tryptophan supplementation promotes cellular reprogramming to iPSCs. Interestingly, this was not related to its role in *de novo* NAD^+ synthesis, but instead to increased kynurenine production, which possibly modulates the Aryl hydrocarbon receptor (Ahr) transcription factor.

Working with liver organoids, Heather Christofk (UCLA, USA) observed that differentiation of liver progenitors is induced by vitamin C, the transporter of which shows increased expression in differentiated hepatocytes. Given the requirement of vitamin C for DNA demethylation by α-ketoglutarate-dependent dioxygenases (Blaschke et al., 2013), it will also be interesting to define a causal relationship between transporter expression, DNA methylation and hepatocyte differentiation in other contexts where α-ketoglutarate-dependent demethylation determines cell identity. On a similar theme, Lydia Finley (Memorial Sloan Kettering Cancer Center, New York, USA) explained how low glutamine oxidation and high α-ketoglutarate production is an intrinsic feature of naïve ESCs. Transient glutamine depletion allows enrichment of naïve pluripotent cells from a heterogeneous culture of metastable mouse ESCs cultured in serum/LIF, thus recapitulating the effect of combined MEK/GSK3b inhibition (2i) (Vardhana et al., 2019). Interestingly, the converse is true in skin epidermal cells, where increased α-ketoglutarate production through *de novo* serine synthesis drives differentiation of serine-starved skin stem cells and squamous cell carcinoma (Baksh et al., 2020). Also working in the skin, Anupama Hemalatha (from the lab of Valentina Greco, Yale University, New Haven, CT, USA) used *in vivo* optical redox imaging of NADH and FAD as a sensitive readout of metabolic changes. Using two models of oncogenic tolerance in skin, she showed that the recovery of the redox ratio reflects on the ability of cells to repopulate the basal layer after initial oncogenic mutations.

We are what we eat: dietary decisions in development and disease

Our diet has a clear impact on our health, and many speakers have studied the impact of nutrition on the development of disease. Jason Locasale (Duke University, Durham, NC, USA) discussed some of the roles that his lab and others discovered for dietary methionine, which provides the carbon unit for methylation. Strikingly, methionine is one of the most highly variable metabolites in human plasma, with part of these variations coming from the diet. His lab previously described how variations in methionine influence the levels of H3K4me3 (Mentch et al., 2015). Their more recent work focused on the role of methionine in H3K36me3 deposition and myoblast differentiation, and the fascinating genetics behind position effect variegation in *Drosophila*.

Many other speakers turned to *Drosophila* as a model to study the impact of diet on development and disease. Alex Gould (Francis Crick Institute, London, UK) presented a high-fat diet (HFD) model of chronic kidney disease in *Drosophila* (Lubojemska et al., 2021). He showed that lipid droplet (LD) accumulation in HFD decreases mitochondrial volume in nephrocytes and compromises endocytosis. Earlier work from his lab (Bailey et al., 2015) and others (Liu et al., 2017) emphasized LDs as emerging players in development and disease, but why they could be either harmful or protective in different contexts remained poorly understood. His lab have now found that, although genetic inhibition of triglyceride synthesis or activation of lipolysis both lead to decreased LDs, only lipolysis activation rescues HFD-related kidney dysfunction. Shuttling fatty acids through LDs before mobilising them for mitochondrial β-oxidation might confer a protective effect that could be exploited as a future therapeutic strategy.

Translating findings from *Drosophila* to humans, Aurelio Teleman (DKFZ, Heidelberg, Germany) presented work following up on earlier findings that high levels of dietary stearic acid (C18:0) could rescue motor deficits in a *Drosophila* model of Parkinson's disease (Senyilmaz et al., 2015). His lab conducted a clinical trial and observed rapid mitochondrial fusion and increased fatty acid β-oxidation in peripheral blood cells upon ingestion of high but physiological levels of stearic acids by healthy volunteers (Senyilmaz-Tiebe et al., 2018). They also identified several candidate proteins that were post-translationally modified by C18:0, providing an intriguing glimpse into possible mechanisms by which metabolites from our diet can directly affect signalling pathways.

Finally, bridging yeast genetics with *Drosophila*, Tadashi Uemura (Kyoto University, Japan) performed an elegant forward genetic screen by feeding wild-type *Drosophila* larvae mutant yeast strains from the yeast knockout collection. This assay allows an assessment of how differences in food composition affect development and ageing. One of the yeast strains fed contained a mutation in an acetyltransferase, leading to increased levels of long- and very long-chain fatty acids. This larval diet reduced the number of emerging adult flies and shortened their lifespan compared with the control diet, possibly by interfering with histone acetylation. It will be interesting to further investigate which other roles metabolites such as specific lipids or methionine may have in the epigenetic regulation of cell fate and signalling, and to determine beneficial doses of various nutrients.

Metabolic compartmentalization and cooperation

Compartmentalization of metabolic pathways provides a powerful way to increase efficiency while also allowing flexibility. Several speakers studied how spatio-temporal control of metabolite production in the nucleus allows metabolic regulation of the epigenome. Kathryn Wellen (University of Pennsylvania, Philadelphia, USA) described how stable isotope labelling of Acyl-CoAs in cell culture coupled with subcellular fractionation (SILEC-SF) can successfully detect predicted compartment-specific changes in Acyl-CoA abundance, e.g. under hypoxia (Trefely et al., 2020 preprint). Interestingly, specific Acyl-CoAs, such as propionyl-CoA, were selectively enriched in the nucleus compared to the cytoplasm, supporting the idea of the nucleus as a distinct metabolic compartment.

A mitochondrial defect can metabolically threaten genomic stability in the nucleus, as shown by Juan Landoni (from the lab of Anu Suomalainen-Wartiovaara, University of Helsinki, Finland). Studying mice with premature ageing due to defects in the mitochondrial DNA polymerase POLG, they observed increased mtDNA replication, resulting in nucleotide sequestration by the mitochondria and nuclear DNA instability (Hämäläinen et al., 2019). These results challenge the previously accepted model that mitochondrial DNA (mtDNA) mutations might drive ageing.

Paula Gutierrez Perez (from the lab of Luisa Cochella, IMP, Vienna, Austria) talked about the role of miR1, a conserved

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muscle-specific micro-RNA. She discovered that miR1 regulates expression of V-ATPase subunits and other enzymes important for mitophagy and autophagy. Loss of miR1 upregulates V-ATPases, and this affects muscle physiology, with decreased Ca^{2+} and ATP levels, problems in cell-cell fusion and strong impairments of the mitochondrial-lysosomal axis. Intriguingly, even though loss of miR-1 upregulates V-ATPase subunits, this causes loss of function of the complex that severely impairs the mitochondrial-lysosomal axis and muscle (Gutiérrez-Pérez et al., 2020 preprint).

Compartmentalization also occurs between organs. A classic example is the Cori cycle in which muscle-derived lactate is used by the liver for gluconeogenesis. But, as in cells, this requires cooperation and communication between compartments. Many groups are taking advantage of the powerful genetics of *Drosophila* to identify novel organism-wide metabolic crosstalk. Norbert Perrimon (Harvard Medical School, Boston, MA, USA) described his recent work on organ wasting (cachexia), a major cause of cancer-associated morbidity and mortality. Using Yorkie-induced intestinal tumours in *Drosophila*, they identified tumour-secreted factors that induce systemic organ wasting through Upd3- and Impl2-mediated inhibition of insulin signalling (Kwon et al., 2015). They are now using innovative proximity-labelling techniques (Chen et al., 2015) and single-nuclei sequencing to identify additional factors. One downstream target of Impl2 is Reptor, which promotes mitochondrial respiration and suppresses glycolysis in muscle. Importantly, Reptor downregulation rescues tumour-induced muscle wasting, thus providing a promising novel therapeutic target for cancer-related cachexia. Building on these proximity-labelling techniques, Wei Wei (from the lab of Jonathan Long, Stanford University, CA, USA) introduced us to bio-orthogonal tags that enable labelling, detection and enrichment of secreted polypeptides in a cell type-selective manner in mice (Wei et al., 2021). Reminiscent of the hijacking of muscle metabolism by *Drosophila* gut tumours, Ayelet Erez (Weizmann Institute of Science, Rehovot, Israel) observed decreased urea synthesis in the liver of cancer patients (Lee et al., 2018). Interestingly, the rewiring of the urea cycle in the liver of mice that had undergone orthotopic tumour transplantation was independent of liver cancer.

Similar to the pH and pyruvate sensors used by the Pourquié and Aulehla labs in vertebrates, genetically encoded sensors to measure concentrations of specific metabolites have also been readily adopted in *Drosophila*. Irene Miguel-Aliaga (Imperial College London, UK) took advantage of glucose and lactate FRET sensors to confirm male-specific bias of glycolytic gene expression in one region of the *Drosophila* intestine (Hudry et al., 2019). When investigating why the gut would have this sex bias in glycolytic gene expression, they noticed a recurrent association between this specific region of the gut and the testes. The male gonad secretes a cytokine that activates JAK-Stat signalling in the neighbouring intestinal cells, causing them to increase glycolysis and citrate production. Interestingly, gut-derived citrate plays essential roles in germline maturation in the testes. Given the stereotypic association of various gut regions with many abdominal organs, not only in *Drosophila* but also in humans, it will be interesting to see whether similar metabolic cooperation might exist elsewhere.

The intestinal microbiome is also recognized as a metabolically active organ, composed of trillions of bacteria, the metabolism and metabolites of which play crucial roles in many physiological and pathological conditions. Wendy Garrett (Harvard School of Public Health, Boston, MA, USA) showed that high dietary sulphur amino acids increase H_2S production by the microbiome, modifying the gut proteome (Lobel et al., 2020). Her lab showed that increased H2S production by the microbiome improves kidney function in chronic kidney patients. Lora Hooper (University of Texas Southwestern Medical Center, Dallas, USA) talked about the crosstalk between microbiome, lipid metabolism, immune cells and circadian rhythm. She showed that the microbiome, through the immune system, regulates the expression of *Nfil3* and *Hdac3* genes in the intestine (Kuang et al., 2019). Those genes are also regulated by the circadian clock and control lipid absorption in the intestine. Given the growing evidence demonstrating crucial roles for the microbiome in regulating not only metabolism but also the progression of many diseases such as cancer and Parkinson's disease, it will be interesting to identify more connections and crosstalk between the bacteria in our intestine and beyond, and the metabolism across our body.

A multi-ome view on metabolic plasticity

The dynamic rewiring of metabolic pathways, either as a cause of physiological or pathological cell fate transitions or as a consequence of environmental or dietary changes, was a recurrent theme throughout many talks. A more comprehensive understanding of how metabolic plasticity occurs at an organism-wide level may lead to personalized precision medicine, by integrating dietary information, metabolomics and high-throughput genomics. Several groups have started tackling this, taking advantage of simpler organisms that allow large-scale perturbations. In an approach similar to Tadashi Uemura's, but using *C. elegans* strains instead of *Drosophila* and feeding them mutant bacteria instead of yeast, Marian Walhout (University of Massachusetts, Amherst, USA) is building large-scale metabolic networks that allow *in silico* prediction of individual responses to genetic and dietary perturbations. Her lab discovered a novel pathway for vitamin B12-independent propionate breakdown that is transcriptionally activated in absence of dietary vitamin B12 (Watson et al., 2016).

Markus Ralser (Francis Crick Institute, London, UK; Charité Universitätsmedizin, Berlin, Germany) used the yeast knockout collection to perform quantitative amino acid metabolomics, as well as new high-throughput proteome technologies, to infer the metabolic roles of all genes across the yeast genome (Messner et al., 2021; Mülleder et al., 2016). This allowed Ralser's lab to identify novel roles for various amino acids, beyond what is required for growth. The uptake of lysine, for example, exceeds the growth requirements up to 100 times, but helps cells to become more oxidant tolerant by significantly increasing NADPH-dependent glutathione levels (Olin-Sandoval et al., 2019). Ralser also described how cell communities share metabolites in order to grow together and how the more the cells cooperate in metabolism, the higher the metabolic ability of the cells and the higher the export activity. Given that the export system of microbes is not very specific, these results provide another mechanism for antimicrobial tolerance.

Technological innovation fuels scientific progress

An expanding toolkit to measure and perturb metabolism in a targeted way has clearly been an important factor in the recent success of the field. Given the spatial and temporal heterogeneity observed during development and disease, the field is moving towards spatio-temporally resolved single-cell metabolomics. Theodore Alexandrov (EMBL, Heidelberg, Germany) presented SpaceM (Rappez et al., 2019 preprint) for single-cell metabolomics through MALDI-imaging mass spectrometry, integrated with microscopy. This allowed his lab to analyse lipid droplet composition in heterogeneous steatotic hepatocyte populations.

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The resolution (10 µm pixel size) currently precludes an analysis of tissue sections and confluent cell cultures, but future advances may address this.

Several groups presented data using stable isotope tracing and flux analysis *in vivo*. Both Ralph DeBerardinis (University of Texas Southwestern Medical Center, Dallas, USA) and Heather Christofk described experiments with ^{13}C-labelled glucose infusion in pregnant mice and subsequent dissection and analysis of foetal organs at different stages of development. The time course experiments conducted by Ashley Solmonson in the DeBerardinis lab, dissecting embryos at different time points from the same pregnant dam were particularly impressive. Close interactions with a clinical program to study inborn errors of metabolism through metabolomics and genomics now allow the DeBerardinis lab to better understand the impact of specific mutations on metabolism. Joshua Rabinowitz (Princeton University, NJ, USA) had previously observed an important role for circulating lactate, rather than glucose, to feed the TCA cycle, although the relative contributions were clearly tissue specific (Hui et al., 2017). Now, he shows that the source of NADPH for *de novo* lipogenesis also differs between tissues. Whereas brown adipose tissue uses glucose through the pentose phosphate pathway for NADPH production, the liver mainly relies on folate-mediated serine catabolism (Fan et al., 2014).

Finally, having started off with the non-enzymatic pH-dependent acetylation of β-catenin in the Keynote address by Olivier Pourquié, the meeting came full circle when Jared Rutter (University of Utah, Salt Lake City, USA) presented his approach to obtain a more comprehensive view on non-enzymatic post-translational protein modifications. His lab previously developed mass spectrometry integrated with equilibrium dialysis (MIDAS) (Orsak et al., 2012), whereby candidate proteins and a library of metabolites are separated by a semipermeable membrane. They have now screened over 200 proteins, identified thousands of novel interactions and found that more than 70% of the metabolite-protein interactions tested affect protein function *in vitro*. Inhibition of one isoform of LDH, LDHA but not LDHB, by long-chain acyl-CoAs is an intriguing example of how tissue-specific allosteric regulation by lipids may confer cell-type-specific signalling roles to dietary nutrients and metabolites.

Concluding remarks

Remote interactions, technological innovation, rewiring of our pathways in response to the environment and compartmentalization: the main themes of this meeting have all become part of our lives in the current age of COVID-19. Many challenges still lie ahead to dissect the role of metabolism in cell fate decisions in time and space, but, as for the current pandemic, the novel tools at our disposal hold a lot of promise for the future. For three young researchers like us, this virtual meeting was a timely reminder of the fascination and highly stimulating interdisciplinary science behind every single theme. It is an exciting time to move from correlative studies towards state-of-the-art mechanistic investigations in the metabolic decisions that accompany and drive development and disease. We look forward to this new era in which metabolism emerges as a driver and not merely a passenger.

Acknowledgements

We are grateful to the organizers for putting together this fantastic group of speakers and maintaining such a high-level meeting, with all the added challenges of the virtual set-up. We are also grateful to all speakers for sharing unpublished results and advancing the field in such a phenomenal way.

Competing interests

The authors declare no competing or financial interests.

Funding

J.v.d.A. is a Wellcome Clinical Research Career Development Fellow (219615/Z/19/Z) and receives support from the Medical Research Council Mitochondrial Biology Unit (MC_UU_00015/10).

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