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SPOTLIGHT

Reclaiming Warburg: using developmental biology to gain insight into human metabolic diseases

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ABSTRACT

Developmental biologists have frequently pushed the frontiers of modern biomedical research. From the discovery and characterization of novel signal transduction pathways to exploring the molecular underpinnings of genetic inheritance, transcription, the cell cycle, cell death and stem cell biology, studies of metazoan development have historically opened new fields of study and consistently revealed previously unforeseen avenues of clinical therapies. From this perspective, it is not surprising that our community is now an integral part of the current renaissance in metabolic research. Amidst the global rise in metabolic syndrome, the discovery of novel signaling roles for metabolites, and the increasing links between altered metabolism and many human diseases, we as developmental biologists can contribute skills and expertise that are uniquely suited for investigating the mechanisms underpinning human metabolic health and disease. Here, we summarize the opportunities and challenges that our community faces, and discuss how developmental biologists can make unique and valuable contributions to the field of metabolism and physiology.

KEY WORDS: Warburg effect, Human disease, Metabolism, Inter-organ communication, Oogenesis, *Drosophila*

Introduction

> *It is, of course, to be hoped that in time the combined attack of the problem of development by genetics and experimental embryology and especially by chemistry may lead to the discovery of the physiological action of genes*.
>
> Thomas Hunt Morgan, 1923

From the late 1800s until the molecular biology revolution in the mid-20th century, a significant number of developmental biologists investigated how metabolism shapes metazoan development. This era of studies and ideas, including Haldane's musings on the biochemical basis of animal body size (Beadle and Ephrussi, 1936; Haldane and Maynard Smith, 1985) and Beadle and Ephrussi's studies of *Drosophila* eye pigmentation (Beadle and Ephrussi, 1936; Haldane and Maynard Smith, 1985), defined our modern understanding of developmental biology and genetics. However, the questions that developmental biologists asked one century ago about the link between animal growth and metabolism remain relevant today (Miyazawa and Aulehla, 2018). Even with limited knowledge of biochemistry, a curious observer intuitively understands that organismal growth and metabolism are intimately linked: it is clear that, by taking nutrients, energy and other cues from the environment, living systems can produce remarkably multifaceted and highly organized forms. This observation highlights the extraordinary complexity of physiological and gene regulatory networks that sense nutrients, orchestrate the energy-intensive funneling of thousands of different molecules into cellular and extracellular structures, and coordinate biosynthetic processes throughout an entire organism. Developmental biologists are ideally suited to understanding exactly how metabolism and cell structure/function are linked in living organisms. In fact, many of the signaling pathways that drive animal metabolism and control how cells and tissues uptake and metabolize various molecules were discovered by our community.

The inseparable relationship between metabolism and genetic factors that control growth and development has broad implications for biomedical research, which has become increasingly focused on the metabolic basis of human diseases. This renewed interest in metabolism illuminates a foundational pillar of developmental biology and raises the question of how our community will push the frontiers of an exciting and rapidly evolving field. Such a position is both privileged and unexpected to those of us trained near the turn of the millennium – a time when developmental biologists were producing a constant stream of studies that described new genes involved in cell-cell communication, signal transduction and gene expression. During this decade or so, our quest to find 'interesting' genes often involved scouring microarray data while systematically ignoring those 'pesky' housekeeping genes ending in words like dehydrogenase, isomerase or reductase. Such a mindset persists even today as most studies of developmental metabolism, with notable exceptions, focus on well-described signal transduction pathways that control the abundance of metabolic enzymes, rarely exploring how changes in metabolic flux themselves shape cellular function during development. Moreover, many metabolism-related studies conducted in model organisms are based upon genes known to cause human metabolic diseases. While these candidate gene

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Advocating developmental biology

This article is part of Development's Advocacy collection – a series of review articles that make compelling arguments for the field's importance. The series is split into two: one set of articles addresses the question 'What has developmental biology ever done for us?' We want to illustrate how discoveries in developmental biology have had a wider scientific and societal impact, and thus both celebrate our field's history and argue for its continuing place as a core biological discipline. In a complementary set of articles, we asked authors to explore 'What are the big open questions in the field?' Together, the articles will provide a collection of case studies that look back on the field's achievements and forwards to its potential, a resource for students, educators, advocates and researchers alike. To see the full collection as it grows, go to: https://dev.biologists.org/content/advocating-developmental-biology.

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approaches are indispensable, they inevitably raise the question of how we, as developmental biologists, will once again push the frontiers of metabolic research. One answer to this question comes from an unlikely source – Otto Warburg, a well-recognized pioneer in the field of cancer biology.

Warburg's name is now synonymous with tumor metabolism, and his discoveries spawned the recent renaissance of studies of metabolism relating to cancer biology (Vander Heiden and DeBerardinis, 2017; Warburg, 1956; Warburg et al., 1924). But before Warburg was trying to cure cancer, before he was a Prussian cavalry officer wounded on the front lines of World War I, and before Albert Einstein personally encouraged him to leave the war and return to academia, he made pioneering discoveries at the intersection of metabolism and developmental biology (Krebs and Schmid, 1981; Otto, 2016). From 1908 to 1914, Warburg would occasionally visit the Stazione Zoologica in Naples, where he examined the metabolism of sea urchin fertilization (Krebs and Schmid, 1981; Otto, 2016). Warburg's key discovery during this time was that sea urchin embryos significantly increase oxygen consumption upon fertilization (Krebs and Schmid, 1981; Otto, 2016; Warburg, 1908). While such a finding may seem unremarkable today – as we would expect embryos to have significantly higher energetic demands than unfertilized eggs – this discovery represented one of the first demonstrations that metazoan growth induces (and perhaps requires) measurable changes in metabolism.

Warburg's sea urchin studies laid the foundation for his subsequent characterization of tumor metabolism. As noted by his student and biographer Hans Krebs: 'The link between this work [sea urchin fertilization] and the later investigations on cancer is obvious: when a normal cell becomes cancerous, it grows excessively, and in 1922 Warburg set out to test whether cancer cells have an increased oxygen consumption' (Krebs and Schmid, 1981). Instead, Warburg discovered a metabolic phenomenon now referred to as 'the Warburg effect', or aerobic glycolysis, which is characterized by elevated glucose consumption coupled to oxygen-independent lactate production, thereby inducing a metabolic state strongly favoring biosynthesis (for reviews, see Hay, 2016; Vander Heiden and DeBerardinis, 2017). This discovery has shaped our current understanding of how metabolism promotes tumor growth, and the Warburg effect remains intensely studied.

While the Warburg effect is primarily associated with tumor metabolism, similar metabolic changes also occur in a variety of normal proliferating cells and growing tissues (Miyazawa and Aulehla, 2018; Sieber and Spradling, 2017). Developmental biologists thus have the opportunity to study 'tumor metabolism' *in vivo* under physiological conditions. This similarity between human disease metabolism and normal metazoan development extends beyond cancer. In fact, many metabolic changes found in disorders such as diabetes and heart failure are also observed in developmental contexts (Dorn et al., 2015; Tennessen and Thummel, 2011). We, as developmental biologists, can use this to our advantage in discovering new metabolic mechanisms that contribute to disease progression. The purpose of this Spotlight, however, is not to review the advances that developmental biologists have made in studying aerobic glycolysis or other metabolic programs, as these have been the recent subject of several excellent reviews (see Miyazawa and Aulehla, 2018; Sieber and Spradling, 2017), but rather to highlight how Warburg's research can guide future studies of developmental metabolism.

Lessons from Warburg: exploring metabolic transitions

Although sea urchin embryos and tumors activate different metabolic programs, it is useful to compare these systems. Warburg's sea urchin studies highlight the power of using a developmental system for studying metabolism; the highly reproducible events surrounding sea urchin fertilization allow for the precise study of a major metabolic transition (Turner and Shapiro, 1988). By contrast, the inherently unpredictable metabolic changes occurring during tumor formation are challenging to study *in vivo*, underscoring why aerobic glycolysis should be studied further in model organisms. By investigating this metabolic switch in a controlled developmental system, such as the mouse tailbud (Bulusu et al., 2017; Oginuma et al., 2017), zebrafish muscle (Tixier et al., 2013), or *Drosophila* embryos and imaginal discs (Tennessen et al., 2014; Wang et al., 2016), we can pinpoint the endogenous mechanisms triggering aerobic glycolysis. Developmental systems provide yet another advantage when compared with tumors because healthy tissues turn off aerobic glycolysis in a controlled manner (White et al., 1999), thus providing an opportunity to examine the endogenous mechanisms that turn off this metabolic program. Developmental metabolism studies will also be invaluable in informing research into how endogenous mechanisms might be co-opted by cancers and therapeutically targeted.

The same rationale for developmental biologists studying the Warburg effect applies to metabolic shifts observed in other chronic diseases, including diabetes, heart failure and neurodegeneration. The mammalian heart, for example, relies largely on glucose and lactate metabolism for fetal energy production but activates fatty acid oxidation at the onset of neonatal development (Makinde et al., 1998). This metabolic switch is dependent on the nuclear receptor ERRγ and the transcriptional co-activator PGC1α, which together activate the expression of genes involved in neonatal cardiac metabolism (Alaynick et al., 2007; Lai et al., 2008). Studying this metabolic transition, however, is not only relevant for treating birth defects but also for understanding heart failure. Many of the metabolic changes that are activated during cardiac development are reversed in the failing heart, as evident by decreased ERRγ and PGC1α expression and reactivation of fetal cardiac metabolism in cardiomyopathies (Dorn et al., 2015). But while heart failure develops over a period of years, neonatal development in model organisms can be easily studied in a controlled manner, providing an important opportunity to explore the metabolic mechanisms underlying this disease.

The above examples illustrate why developmental biologists should press our advantage in studying metabolic regulation. From studies of the heterochronic pathway in *C. elegans* to nuclear receptor signaling in *Drosophila* and amphibians, we have a rich history of developing the experimental techniques and tools required to precisely study developmental transitions (Denver, 2013; Ou and King-Jones, 2013; Rougvie and Moss, 2013). This same expertise should be applied to studying metabolism, with the overarching goal of understanding the metabolic changes underlying human disease.

More lessons from Warburg: emerging technologies can provide new opportunities

Warburg's pioneering studies of cancer metabolism resulted directly from his thoughtful experimental design and extraordinary technical ability. As noted by Krebs, Warburg showed 'exceptional skills in selecting the right kind of material and in perfecting experimental techniques' (Krebs and Schmid, 1981; Otto, 2016). Warburg's approach provides key lessons for modern studies of developmental metabolism. First, many of Warburg's discoveries were made possible by techniques that he pioneered in spectrophotometry and manometry (Krebs and Schmid, 1981), which provided novel opportunities to measure both small molecules and the production

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and consumption of gasses, respectively. These tools allowed Warburg to ask experimental questions that were previously inaccessible and led to major breakthroughs in our understanding of cancer metabolism.

Metabolic research is again experiencing a technological revival that is providing developmental biologists with the tools necessary to study metabolism with unparalleled precision. The powerful combination of metabolomics and small-molecule sensors allows the visualization of metabolites within individual cells/tissues (Cox et al., 2017; Miyazawa and Aulehla, 2018). For example, a FRET-based sensor for pyruvate led to the discovery of a posterior-to-anterior gradient of glycolytic activity during somite formation within the mouse tailbud presomitic mesoderm (Bulusu et al., 2017). Similarly, the use of a citrate FRET sensor in *Drosophila* was crucial to understanding how the transport of citrate from the intestine to the testis serves a key role in sperm maturation (Hudry et al., 2019). The use of FRET-based sensors is complemented by advances in mass spectrometry that allow for direct visualization of individual metabolites within single cells and can potentially combine metabolomics with cell-specific tools commonly used by developmental biologists (Rappez et al., 2019 preprint). New enhanced methods for measuring metabolic flux are also benefiting developmental biology (Jang et al., 2018). The metabolism and physiology communities have developed exquisitely sensitive techniques for measuring parameters such as oxygen consumption, carbon dioxide production and heat flow, which have the potential to illuminate entirely new phenomena. For example, recent studies of mouse and zebrafish embryos using metabolic flux analysis and isothermal calorimetry, respectively, revealed unexpected links between metabolism and embryonic development (Chi et al., 2020; Rodenfels et al., 2019). The widespread adoption of such tools by our community will be essential for investigating how metabolism is regulated *in vivo*.

Warburg's sea urchin studies also emphasize the importance of thoughtfully selecting an experimental system best suited for studying a specific metabolic transition/switch. Warburg chose to measure oxygen consumption in sea urchin embryos because 'development of the fertilized egg is very rapid so that…much happens in a short time' (Krebs and Schmid, 1981). With this quote in mind, our community should embrace emerging model organisms that are ideal for examining particular metabolic processes. After all, life evolved under selective pressures that included extreme environmental and nutritional stresses, thus adapting to many of the metabolic challenges that are now associated with human disease. Adaptive thermogenesis, e.g. where 'beiging' of white fat increases metabolic rate, leads to weight loss and improves insulin sensitivity, and is of particular interest to research aiming to treat obesity and diabetes (Ikeda et al., 2018). A key feature of adaptive thermogenesis is elevated mitochondrial uncoupling, which allows protons to re-enter the mitochondrial matrix independently of ATP synthase and release energy as heat (Lowell and Spiegelman, 2000). Although mitochondrial uncoupling can be studied in standard model systems, the wax moth, *Galleria mellonella*, is a potentially exciting model. Its larvae can burn superfluous dietary lipid to generate large quantities of metabolic water, while the extra calories become heat (Jindra and Sehnal, 1989). This adaptive trait, which involves mitochondrial uncoupling, allows *G. mellonella* to thrive under waterless culture conditions and could provide the biomedical community with a new perspective on how this metabolic process is controlled *in vivo*. Similar examples of how animal metabolism adapts to extreme environments can be found throughout the last century of research on animal physiology and, as new technologies facilitate genetic studies in non-canonical model systems, the potential for discoveries tying metabolism to development and physiology *in vivo* will significantly expand.

Beyond Warburg: investigating metabolic dysfunction at the organismal level

Warburg's studies of aerobic glycolysis used tumor slices incubated in an artificial medium – the most logical and powerful approach available at the time (Warburg et al., 1924). However, Warburg's approach, much like modern cell culture studies, failed to replicate the complexities associated with an *in vivo* metabolic environment, in which cells are exposed to a milieu of endocrine signals and circulating small molecules. The limitations of such *in vitro* approaches were recently highlighted by multiple stable isotope tracer experiments using ^{13}C-labeled glucose in individuals with cancer (Courtney et al., 2018; Faubert and DeBerardinis, 2017; Hensley et al., 2016). These studies revealed the complexity of cancer metabolism, with different tumor types consuming and using glucose in distinct manners. Some tumors, such as lung cancers, do not activate the Warburg effect, but rather rely on glucose oxidation and consume lactate (Faubert et al., 2017). Indeed, mitochondrial metabolic pathways, which are often ignored by oversimplified descriptions of the Warburg effect, play an essential role in tumor metabolism *in vivo* (DeBerardinis and Chandel, 2020; Martinez-Reyes and Chandel, 2020). Moreover, simple models of cancer metabolism fail to convey the cellular and metabolic heterogeneity within individual tumors, where metabolic flux differs among cell types (e.g. malignant cells, stroma, immune cells and cancer stem cells) and metabolites can be transferred among cells within a microenvironment (Kim and DeBerardinis, 2019). This high complexity and variability of cancer metabolism underscores the importance of studying metabolism *in vivo*.

The problems associated with *in vitro* studies of metabolism are not limited to cancer cells. Any isolated cell or tissue that is incubated in artificial media will inevitably generate metabolic artifacts; therefore, metabolic research is best conducted *in vivo*. In this regard, developmental biologists have distinct advantages. The growth and development of any multicellular organism requires the coordinated synthesis, storage and transport of metabolites between cells. The metabolic needs of individual cells and tissues must be balanced across the entire organism through complex metabolic networks. Having spent decades generating the tools required for cell- and tissue-specific studies, our community is poised to probe the physiological, cellular and molecular mechanisms that coordinate metabolism across an entire living organism.

Studies of adult *Drosophila* oogenesis illustrate how we can leverage existing tools to investigate the systemic regulation of metabolism. Oogenesis is an energy- and nutrient-intensive process involving the massive accumulation of lipids, carbohydrates and other macromolecules in the oocyte, in tight coordination with its complex development from an undifferentiated precursor into a mature oocyte. Developmentally controlled metabolic changes occur at multiple steps, ending with entry of the oocyte into a quiescent state (Sieber et al., 2016). A multi-organ physiological network further coordinates the developmental control of oogenesis with the metabolic state of the organism (reviewed by Drummond-Barbosa, 2019). Abundant dietary nutrients promote oogenesis through brain-derived insulin and other systemic factors, while adversities trigger downregulation of oogenesis at many stages, thus safeguarding organismal resources (reviewed by Drummond-Barbosa, 2019). Other organs also support the nutritional demands of oogenesis.

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The midgut dramatically expands under a rich diet, thus enhancing nutrient absorption (O'Brien et al., 2011). Mating also leads to enlargement of the adult female midgut and altered enterocyte lipid metabolism to favor egg production (Reiff et al., 2015). Incidentally, mating also stimulates female germline stem cell proliferation through neuropeptide F from midgut enteroendocrine cells (Ameku et al., 2018). Lipophorin-mediated transport of lipids is crucial for oocyte yolk uptake (Matsuoka et al., 2017; Parra-Peralbo and Culi, 2011), while several adipocyte metabolic pathways have specific effects earlier in oogenesis (Matsuoka et al., 2017). Thus, the coordination of hormones, nutrients, metabolism and oogenesis developmental transitions integrates environmental cues and extensive inter-organ communication (reviewed by Drummond-Barbosa, 2019; Weaver and Drummond-Barbosa, 2019).

Research on the *Drosophila* ovary and other highly metabolically demanding tissues/organs, such as the *C. elegans* germline (reviewed by Hubbard and Schedl, 2019), is instrumental for dissecting how the regulation of metabolic flux intrinsically and in peripheral tissues supports the transition of cells through distinct developmental stages with varying metabolic demands in a complex living organism. Notably, the link between tissue development/maintenance and whole-body physiology is widely conserved, including in other tissues with less extreme intrinsic metabolic demands, such as the *Drosophila* midgut (see above, reviewed by Colombani and Andersen, 2020) and developing brain (reviewed by Otsuki and Brand, 2020), among others (Shim et al., 2013).

Developmental biologists are also ideally situated to explore a second emerging focal point of systemic metabolic regulation directly relevant to human diseases but difficult to study *in vitro*. Unlike cultured cells, a developing organism must be able to adapt its growth and maturation to environmental stressors such as nutrient deprivation, toxic compounds and temperature shifts, thus displaying a remarkable level of metabolic plasticity (Gilbert and Epel, 2015; Sieber and Spradling, 2017; Watson et al., 2015). The ability of a cell or tissue to withstand metabolic insults is key to the field of cancer metabolism, which has become increasingly focused on identifying metabolic enzymes that are essential for tumor growth (Vander Heiden and DeBerardinis, 2017). But just as a developing organism adapts to metabolic insults, so too will a tumor evolve to overcome a metabolic inhibitor designed to disrupt cell proliferation and growth. In this context, developmental biologists have a long history of uncovering metabolic mechanisms that impart robustness on development (for examples, see Watson et al., 2014; Watson et al., 2016). Such studies can illuminate compensatory metabolic networks that are directly relevant to studies of tumor metabolism, as a recent study of *Drosophila* Lactate Dehydrogenase (LDH) illustrates. *Drosophila* larvae exhibit very high levels of LDH activity (Rechsteiner, 1970), suggesting that larval metabolism depends on lactate production to maintain redox balance and promote glycolysis. *Ldh* mutant larvae, however, grow at a normal rate because larval metabolism adapts to the loss of LDH activity by synthesizing excess glycerol-3-phosphate (G3P) – a metabolic reaction that also maintains redox balance (Li et al., 2019). These findings reveal uncanny similarities between rapidly growing larvae and tumors, and are directly relevant to studies of cancer metabolism. Specifically, tumors also exhibit elevated LDH-A activity, and LDH-A has thus been proposed as a therapeutic target for inhibiting tumor growth; however, cancer cells synthesize excess G3P in response to LDH-A inhibition (Boudreau et al., 2016), which could potentially render them resistant to LDH-A treatment. Therefore, studies of *Drosophila Ldh* hint at a mechanism by which tumors could become resistant to LDH-A inhibitors and unequivocally demonstrate how studying metabolic plasticity in developmental systems can inform clinical decisions in treating cancer and other human metabolic diseases.

Conclusions

The biomedical community has turned its attention towards studying the metabolic basis of human disease. Many of these efforts focus on metabolic mechanisms that are also active during metazoan development. Our community should therefore exploit our tools and resources to further explore aspects of metabolic regulation that are relevant to disease progression but difficult to study in cultured cells and human patients. The wealth of fundamental information we can generate will no doubt have the power to uniquely inform and propel studies into metabolic disease mechanisms and effective therapies, thereby maximizing the use of research resources and aiding scientific progress and the promotion of human health.

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Competing interests

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