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Measuring the Energetic Costs of Embryonic Development

Peter J. Foster,[1] Manuel Razo-Mejia,[2] and Rob Phillips[2,3,*]
[1]Physics of Living Systems, Department of Physics, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[2]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA
[3]Department of Physics, California Institute of Technology, Pasadena, CA 91125, USA
*Correspondence: phillips@pboc.caltech.edu
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What are the thermodynamic costs of development? In this issue of *Developmental Cell*, Rodenfels et al. (2019) demonstrate that the high energetic cost of coordinated cell division that is regulated by phosphosignaling gives rise to a measurable periodicity in the heat dissipated during zebrafish embryogenesis.

Living organisms are metabolically active, open systems that constantly exchange matter and energy with the world around them. Our quantitative understanding of these exchanges began to take the modern form we would recognize today through the labors of the great pneumochemists like Joseph Priestley and Antoine Lavoisier, who made measurements with primitive devices of the mass and energy transfer of key biochemical reactions between living organisms and the air around them. The kind of deep insights that emerged from these careful quantitative observations included the stoichiometrically-correct equation for photosynthesis (Rabinowitch and Govindjee, 1969). In the tradition of these famed experiments, in work reported in this issue of *Developmental Cell*, Rodenfels et al. (2019) have undertaken a systematic investigation of the heat liberated by zebrafish embryos during development, yielding the beautiful and surprising observation of stereotyped oscillations that are tied to the cell cycle itself (Figure 1).

For all three of us, our delightful first exposure to this experiment took place at the Physiology Course at the Marine Biological Laboratory, where the authors and students in the course built on earlier versions of the measurements of heat flow between developing zebrafish embryos and the surrounding medium in a repurposed calorimeter. This proof-of-concept experiment already revealed the stunning stereotyped oscillations now reported in the current paper. These preliminary empirical observations were built upon and followed up with clever and careful experiments that rigorously explored the phenomenon and, combined with quantitative thinking, provide deep insights into cellular energetics in the context of early development.

In order to make these discoveries, the authors placed synchronized zebrafish embryos inside a calorimeter, and then observed that the dissipated heat from the embryos increased over time (Figure 1). More surprisingly, the authors noticed persistent oscillations in the heat dissipation profile; these oscillations showed a similar period to the known, synchronous division time for the early stages of zebrafish development. To test this connection, they explored the temperature dependence of the oscillation period. They found that the oscillation period scales inversely with temperature in the same manner as the known temperature dependence of the cell-cycle period. Other experiments included the use of desynchronized embryos, which abolished the oscillations, reinforcing the link between the oscillations and the cell cycle. The authors also collected embryos from the calorimeter during different parts of the oscillation and examined their cell-cycle state by DNA staining after fixation. These experiments led to another surprise—instead of peaking at S-phase, when DNA is replicated, or at M-phase, when the cells are dividing, the oscillations had their maximum amplitude somewhere in between, at the S-to-M-phase transition. These experiments suggested that it is not the processes of DNA replication or spindle assembly that lead to the peak in heat dissipation but rather something happening at mitotic entry. Through a combination of assessing cell cycle regulators via immunoblotting and specific cell-cycle inhibitors, the authors show that this something is signaling, driven by phosphorylation by Cdk1 and dephosphorylation by PP2A.

All of these clever experiments pointed to the idea that the energetic cost of Cdk1-dependent signaling could drive the oscillations in the heat-dissipation profile. We find this intriguing because of recent efforts to assess the overall energy budget of a variety of different cell types which point to protein synthesis as the primary energy cost (Lynch and Marinov, 2015). For signaling to explain the observed oscillations, the energetic cost of other processes would have to be significantly smaller than the measured heat dissipation, or these processes would have to take place uniformly during the cell cycle. This might be reasonable in developing zebrafish embryos, which lack traditional growth phases during cleavage divisions and maintain an approximately constant volume. To explore the hypothesis that the cost of signaling drives the oscillations, the authors used a chemical kinetics model to perform simple estimates, which reinforced the argument that this signaling process could give rise to oscillations in heat flow with a magnitude and period consistent with the measured data. Finally, the authors repeated the calorimetry measurements after treating embryos with the microtubule-depolymerizing drug nocodazole, which prevents the embryos' normal cycling through rounds of spindle assembly, cytokinesis, and DNA replication. In these treated embryos, the oscillating heat dissipation profile persisted, lending further evidence that the cost of Cdk1/PP2A-dependent

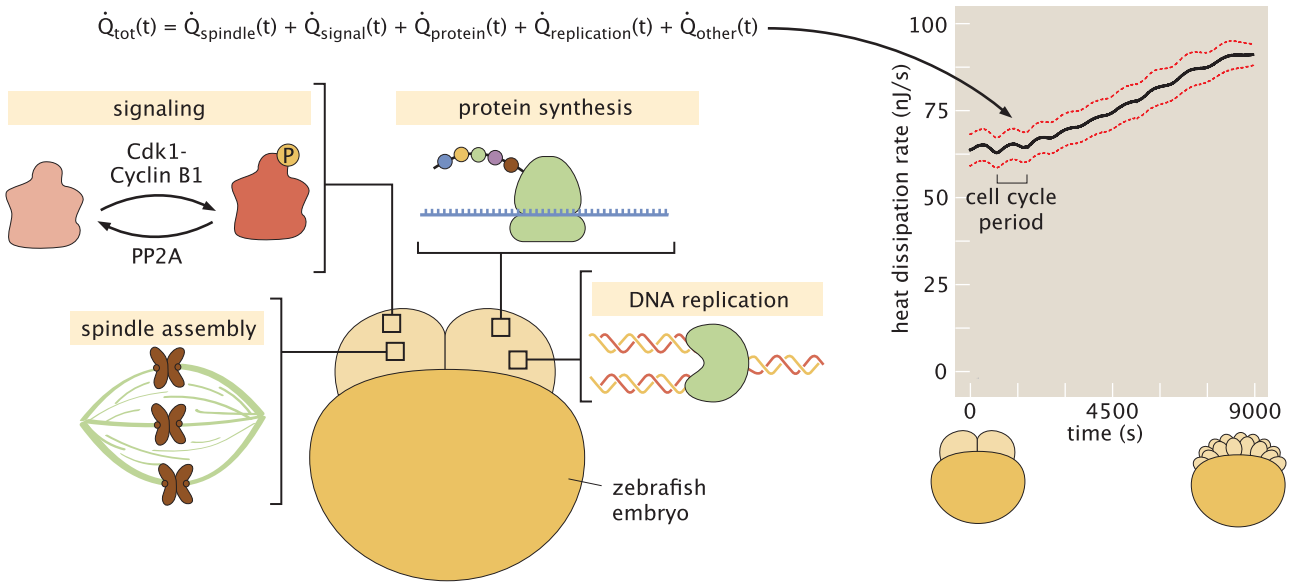

Figure 1. Embryogenesis Meets Calorimetry
In the cell, biochemical processes, such as those of the central dogma, chromosome segregation, and signaling, each incur a free energy cost. By measuring the heat liberated during the embryonic development of zebrafish, Rodenfels et al. (2019) were able to dissect the distinct roles of different processes and show that cellular signaling causes a stereotyped, cell-cycle-triggered oscillation in the dissipated heat.

signaling, and not the costs of replicating the genome or undergoing cell division, is what drives the oscillations.

In our view, these experiments and their corresponding theoretical interpretation should be viewed as part of a larger endeavor in our study of the living world to put the complex processes of living organisms on a strict and rigorous quantitative footing. In his famed book "What is Life?", Erwin Schrödinger's early chapters mused on the question of how genetic information is passed from one generation to the next (Schrödinger, 1944). The birth of molecular biology and, in particular, the discovery of the structure of DNA definitively answered this question. But in the latter parts of his book, Schrödinger then asks about an equally interesting and important question focusing on the physicochemical processes that give living organisms their apparent vitality. In a playful turn-of-the-millennium opinion piece, Kirschner, Gerhart, and Mitchison (Kirschner et al., 2000) called out the importance of exploring the "molecular vitalism" that makes cellular processes work by connecting biological complexity and organization with the underlying physicochemical nature of living systems. This important and interesting paper from Rodenfels et al. (2019) is exactly the kind of response needed to answer that challenge. Calorimetry measurements provide a holistic perspective on cellular energetics by providing a measure of the net heat liberated by all of the biochemical reactions taking place in an organism. By combining these measurements with specific perturbations, the energetic costs associated with a given cellular process can be ascertained. Carrying out this program, Rodenfels et al. (2019) revealed the surprisingly large cost associated with signaling during mitotic entry. Future experiments in this vein will aid in quantifying the cellular energy economy and provide a great step towards understanding the thermodynamics of living systems.

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