Control analysis, mitochondrial bioenergetics and programmed cell death: the Krogh principle in practice

Raul K. Suarez
Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, California 93106-9610

FOR HALF A CENTURY, comparative physiology has been profoundly influenced by the Nobel laureate August Krogh’s dictum (4) that “for a large number of problems, there will be some animal of choice with which it can be most conveniently studied.” As one who did research on insect respiration, Krogh would have greatly admired the work of Chamberlin (1) appearing in this issue of the American Journal of Physiology-Regulatory, Integrative and Comparative Physiology for its use of insects, cutting-edge concepts and techniques, and for addressing fundamental questions that lie at the crossroads of multiple disciplines.

During the transition from larval caterpillar to pupa, the midgut of the tobacco hornworm (Manduca sexta) undergoes programmed cell death (PCD) and is replaced by a pupal epithelium. The midgut is a highly active, aerobic, ion-transporting tissue with abundant mitochondria. It is rather unusual, though, because although inhibition of aerobic ATP synthesis with cyanide causes inhibition of ion transport, inhibition of active ion transport does not affect tissue respiration (5). This is despite the tight coupling between respiration and oxidative phosphorylation demonstrable using isolated midgut mitochondria (2). Thus oxidative ATP synthesis in the midgut displays an apparent insensitivity to control by the rate of ATP hydrolysis. Both ion transport and respiration rates of the midgut decline as PCD proceeds. To elucidate the processes that underlie the decline in respiration rates, Chamberlin used top-down control analysis, a method developed by Martin Brand and colleagues at Cambridge, UK (3). The result is the first paper to report what happens to mitochondrial respiratory control during PCD as well as the application of metabolic control analysis to the study of respiratory control in insect mitochondria.

Mitochondria use energy derived from substrate oxidation to generate a proton electrochemical gradient or protonmotive force, $\Delta p$, that is used to drive ATP synthesis. The top-down control analysis conducted in this work involves conceptually subdividing mitochondrial reactions into blocks that either generate (i.e., substrate oxidation system) or dissipate (i.e., proton leak and phosphorylation system) the $\Delta p$. The kinetic responses of these blocks, measured as $O_2$ consumption, to changes in their common intermediate, $\Delta p$, are determined. The $\Delta p$ is defined by electrical and concentration terms as $\Delta p = \Delta \psi - (2.303RT/F)\Delta \text{pH}$, where $\Delta \psi$ is the membrane potential (cytoplasm - matrix) and $\Delta \text{pH}$ is the difference in pH (cytoplasm - matrix). Chamberlin conducted experiments in the presence of the ionophore nigericin to collapse $\Delta \text{pH}$, so all of $\Delta p$ was expressed as $\Delta \psi$. With the use of the lipophilic cation methyltriphenylphosphonium (TPMP$^+$) and a TPMP$^+$-sensitive electrode to monitor the external concentration of TPMP$^+$, $\Delta \psi$ was calculated using the Nernst equation: $\Delta \psi = (2.303RT/F) \log([\text{TPMP}^+\text{matrix}] / [\text{TPMP}^+\text{external}])$.

In the presence of oligomycin to inhibit ATP synthase, the response of proton leak was monitored as the effect on $O_2$ consumption rate as $\Delta \psi$ decreased in response to inhibition of succinate dehydrogenase with malonate. Again, in the presence of oligomycin, the response of the substrate oxidation system to decreasing $\Delta \psi$ was monitored in response to titration with the uncoupler, FCCP. Finally, under conditions that allow oxidative phosphorylation to proceed, the effect of succinate dehydrogenase inhibition by malonate on $O_2$ consumption was measured to determine the response of the phosphorylation system.

Chamberlin found that under both phosphorylating (state 3) and nonphosphorylating (state 4) conditions, mitochondria show declining respiration rates, expressed per milligram mitochondrial protein, as commitment to metamorphosis and PCD proceed. Control of state 4 respiration is shared by both proton leak and the substrate oxidation system. However, as PCD proceeds, control by proton leak declines whereas control by substrate oxidation over respiration increases. In state 3, 80% or more of the control of respiration is accounted for by the substrate oxidation system, whereas the phosphorylation system accounts for 20% or less and proton leak accounts for a negligible fraction of control. This low level of control by the phosphorylation system on the rate of respiration, as well as the high level of control by substrate oxidation on the phosphorylation system, are unusual properties that may partly explain the observed lack of control by rates of ATP-dependent active ion transport on tissue respiration (5). The high level of control exerted by the substrate oxidation system on respiration in both states 3 and 4 suggests that a decline in the capacity for electron transport may account for the depression of aerobic metabolism during midgut PCD. This was supported by spectroscopic measurements using mitochondrial preparations from various stages that revealed loss of cytochrome $c$, followed by loss of cytochrome aa$_3$ as PCD progressed. It is thought that the precisely timed and orchestrated events leading to midgut PCD are initiated by a pulse of ecdysteroids in the larval hemolymph.

This unique and remarkable quantitative dissection of temporal changes in the control of mitochondrial respiration during apoptosis shall undoubtedly serve as a springboard for further research in several possible directions. But it also dramatically demonstrates how Krogh’s legacy lives on in 21st century comparative physiology.

REFERENCES