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The bioenergetic landscape of respiratory flux control: mapping the scope of metabolic flexibility and mitochondrial fitness

Erich Gnaiger

Oroboros Instruments, Innsbruck, Austria – erich.gnaiger@oroboros.at

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Respiration of living cells studied with the coupling-control protocol (CCP) provides information on cellular bioenergetics and mitochondrial function. Routine respiration R is under control of aerobic ATP demand of the cell within limits of mitochondrial performance. ATP production is inhibited in leak respiration L and decoupled from electron-transfer (ET) for measurement of ET capacity E [1,2]. Normalization of respiratory rates within the coupling-control protocol yields four complementary respiratory flux control indices [4]:

coupling efficiency, $j_{E-L} = (E-L)/E$ — R-ATP coupling ratio, $j_{RLE} = (R-L)/E$

$E-R$ excess ratio, $j_{E-R} = (E-R)/E$ — R-ATP control efficiency, $j_{R-L} = (R-L)/R$

E is not a surrogate for OXPHOS capacity P which requires ATP production stimulated by kinetically saturating ADP concentrations in permeabilized cells [3]. Consequently, $E-R$ excess capacity must be distinguished from the actual OXPHOS reserve capacity, $P-R$. Although derived from only two independent flux control ratios, L/E and R/E, these indices provide complementary views of the bioenergetic landscape. Bioenergetic cluster analysis (BCA) maps mechanistically distinct trajectories through this landscape [4].

Flux control models distinguish changes of coupling efficiency driven by leak respiration ($L\uparrow$; coupling model) or ET capacity ($E\downarrow$; capacity model). Compensatory changes maintain specific respiratory functions until a breakpoint is reached, beyond which further advancement requires adjustment of controlled bioenergetic variables [4]. The extent of change of the controlling rate defines advancement along trajectories up to breakpoints that limit the scope of metabolic flexibility (**Figure 1a,b**). An increase in leak respiration can initially be compensated by increasing routine respiration, maintaining ATP turnover despite declining coupling efficiency. Once the capacity for compensation is exhausted, further dyscoupling compromises ATP-coupled respiration (coupling model; **Figure 1a**). Conversely, declining ET capacity may initially remain without any effect on routine respiration until $E-R$ excess capacity is exhausted (capacity model; **Figure 1b**). Hence, identical changes in coupling efficiency may reflect fundamentally different underlying mechanisms which can be distinguished by BCA (**Figure 1c**).

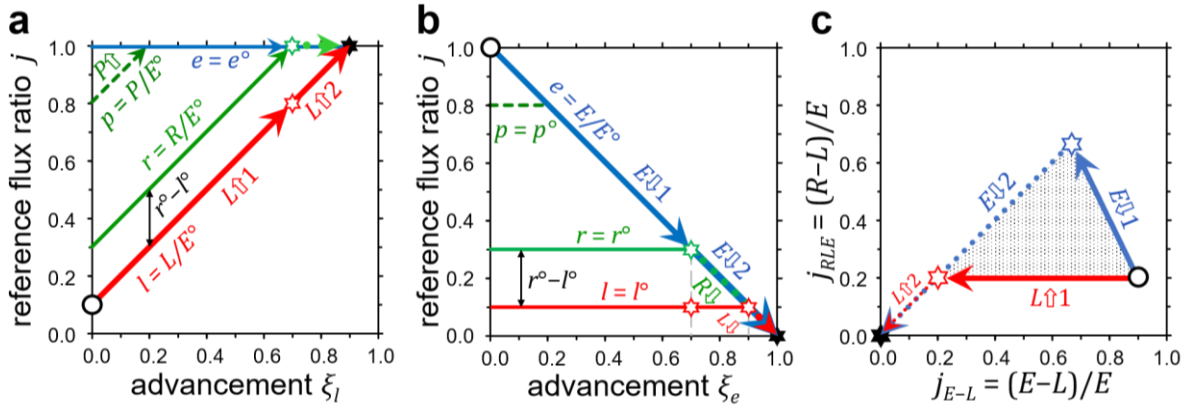

Figure 1. Mapping the scope of respiratory control indices. Trajectories (arrows) to breakpoints (asterisks) from control or initial states (circles): $l° \stackrel{def}{=} L°/E° = 0.10$; $r° \stackrel{def}{=} R°/E° = 0.30$; $p° \stackrel{def}{=} P°/E° = 0.80$. **(a)** Advancement of leak ξ_l. **(b)** Advancement of ET-capacity ξ_e. **(c)** R-ATP coupling ratio, $j°_{RLE} = 0.20$;initial coupling efficiency $j°_{E-L} = 0.90$. Red arrows: coupling model ($L⇧$). Blue arrows: capacity model ($E⇩$). Shaded area indicate the data space between the coupling and capacity models (after [4]).

The direction of a bioenergetic change alone does not necessarily distinguish mitochondrial dysfunction from adaption. Physiological *adaption* within an organism is distinguished from evolutionary *adaptation* across generations [5]. A decrease in mitochondrial density or coupling efficiency may represent adaption within a range of metabolic flexibility, whereas progression beyond a tipping point indicates emerging mitochondrial dysfunction.

BCA thus moves interpretation beyond isolated increases or decreases in respiratory variables toward mechanistic evaluation of mitochondrial fitness. Respiratory control indices characterize mitochondrial quality but must be complemented by respiratory capacities when mitochondrial quantity changes. Implementation of BCA is an important step toward mapping the scope of metabolic flexibility for evaluation of mitochondrial fitness with quality-controlled diagnostic standards [4].

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