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Combining fluorescence and luminescence to investigate the cell biology of the mitochondrial inhibitory protein, IF-1

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In our attempts to understand the factors that determine the cellular responses to impaired mitochondrial function, we wish to explore the relationships between ATP homeostasis and other aspects of mitochondrial and cell (patho)physiology – mitochondrial potential and redox state, cellular calcium and pH. Currently, the only available assay for intracellular ATP in living cells requires transfection of cells with luciferase and measurement of the luminescence signal from the cells in the presence of luciferin. In order to relate changes in [ATP] to other cellular variables which are readily measured using standard fluorescence indicators, we have devised an instrument that allows near simultaneous measurement of luminescence from a population of cells interleaved with measurements of fluorescence from a small subsample of cells. The instrument consists in essence of a light tight enclosure containing epi-fluorescence illumination through a water immersion objective coupled to an inexpensive low light level digital camera (Rolera XR from Q Imaging). Immediately beneath the coverslip of cells lies a photon counting PMT (Hamamatsu H6180-01). Shutters in front of the light source and in front of the PMT protect the PMT from the epi-illumination, and software controlled triggers control the timing of image acquisition, shutter regulation and data acquisition from the PMT. In this way, we are able to measure luminescence signals from cells transfected with luciferase (or aequorin) while monitoring changes in mitochondrial potential (rh123 or TMRM), in calcium (fluo-4, fura-2, rhod-2) or in NADH autofluorescence etc. We have called this instrument a Fluminometer or fluoro-luminometer.

We have employed this system in particular to explore the cell biology of the mitochondrial inhibitory factor (IF-1). This is a mitochondrial protein of 84 amino acids, which binds the β-subunit of the F1 component of the F1-F0–ATP synthase, limiting its hydrolytic activity and protecting the cell from ATP depletion when mitochondrial function is compromised. While the F1-F0–ATP synthase is responsible for the ATP synthesis needed for normal cellular homeostasis, it is a proton motive ATPase driven as an ATP synthase by the proton motive force (mitochondrial membrane potential). In response to a fall in mitochondrial potential, such as occurs in ischemia, it switches from ATP synthesis to ATP hydrolysis, potentially transforming mitochondria in ATP consumers. IF-1 counteracts this event acting as a non competitive inhibitor of the ATPase. We have imaged the intracellular localization of the protein and monitored its expression level among various tissues in relation to the β-subunit of the F1-F0–ATP synthase. We are also exploring the ability of IF-1 to modulate mitochondrial function during inhibition of mitochondrial respiration. To this end, IF-1 expression levels have been genetically manipulated and the mitochondrial parameters investigated. IF-1 activation was promoted either using CN to inhibit mitochondrial respiration or by addition of uncoupler, while mitochondrial membrane potential ($\Delta\psi$) and ATP levels are monitored as described above. The combination of these signals should extend our understanding of the relationships between changes in mitochondrial membrane potential, intracellular calcium and ATP homeostasis in intact cells.

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