HHS Public Access

Author manuscript

Kidney Int. Author manuscript; available in PMC 2025 September 01.

Published in final edited form as:

Kidney Int. 2024 September ; 106(3): 362–364. doi:10.1016/j.kint.2024.06.019.

See the power in kidney cells with ATP biosensor

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Abstract

Yamamoto *et al.* developed an exciting technical advance to examine intracellular adenosine triphosphate levels with single-cell resolution in intact living kidney tissue, including in tubular and vascular segments that lie deep under the kidney surface. The work is a significant advance on prior *in vivo* biosensor studies, and it allows for mechanistic investigation of alterations in cell metabolism, kidney disease pathobiology, and the effects of drug treatments on energy sources in different kidney cell types.

The kidneys are well known for their enormously high level of energy demand for performing normal physiological functions, including tubular ion transport processes. In the cells' powerhouse, mitochondria generate adenosine triphosphate (ATP) from oxidative phosphorylation (OXPHOS), whereas in the cytosol, ATP is produced from glycolysis. In addition to powering healthy kidney function, ATP dynamics also have key importance in the development of renal pathologies. Altered energy metabolism in mitochondrial dysfunction and damage have been implicated in the pathobiology of acute kidney injury (AKI) due to either ischemia or nephrotoxic drugs.[1] Changes in cell metabolism also are at the heart of the mode of action of recently emerging renoprotective drugs and drug combinations, including sodium-glucose cotransporter-2 inhibitors and glucagon-like peptide-1 receptor agonists. Therefore, insights into the intensity and dynamics of ATP production in different renal cell types are essential for gaining an improved understanding of normal kidney function and the development of kidney disease, and for drug development.

Multiphoton microscopy (MPM) is an ideal research technique to study renal physiology, pathophysiology, and therapeutics, including mitochondrial and intracellular ATP dynamics due to the well-known cellular heterogeneity of renal energy metabolism, and dynamic metabolic processes with single-cell and subcellular (organelle) resolution in intact living kidney tissues.[2] MPM imaging of real-time changes in mitochondrial structure and function

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DISCLOSURE

JP-P and GG are cofounders of Macula Densa Cell LLC, a biotechnology company that develops therapeutics to target macula densa cells for a regenerative treatment for chronic kidney disease. Macula Densa Cell LLC has a patent entitled "Targeting macula densa cells as a new therapeutic approach for kidney disease" (US patents 10,828,374 and 11,318,209).

in response to ischemia-reperfusion injury or drug toxicity has been performed in rodent kidney slices *ex vivo*[3] or in intact kidneys *in vivo*,[4] using endogenous (e.g., nicotinamide adenine dinucleotide [NADH] and flavin adenine dinucleotide [FAD]) and exogenous fluorophores (e.g., tetramethyl rhodamine methyl ester [TMRM] or MitoTracker dyes to measure mitochondrial membrane potential, and dihydroethidium to detect reactive oxygen species). Recently, MPM imaging of GO-ATeam2 mice expressing genetically encoded, fluorescence resonance energy transfer–-based ATP biosensor successfully visualized intracellular, spatiotemporal ATP dynamics at the single-cell level.[5] These studies established that proximal tubule (PT) cells' energy metabolism has higher sensitivity to ischemia, as evidenced by rapidly dissipating mitochondrial membrane potential and decreased ATP levels, and the slow recovery rate of ATP upon reperfusion[3-5] In contrast, cells of the distal tubule (DT) featured better-maintained mitochondrial membrane potential, gradually decreased ATP levels, and a fast rebound rate of ATP upon reperfusion.[3-5] However, MPM imaging of the intact living kidney *in vivo* currently is limited to the initial 100–150 μm beneath the kidney surface, meaning that the outer medullary tubule segments (S3 proximal tubule, thick ascending limb of the loop of Henle) that are important players in AKI pathogenesis are out of reach with this technology.

The novel system developed by Yamamoto *et al.*[6] represents a significant advance, compared with prior MPM imaging studies, and enabled visualization of intracellular ATP dynamics in various kidney cells in living kidney tissue, including deeper nephron segments. This new technical advance uses MPM imaging of a kidney slice-culture system expressing the previously developed GO-ATeam2 fluorescence resonance energy transfer-based ATP biosensor to quantitatively visualize ATP dynamics derived from either OXPHOS or glycolysis pathways in PT and DT nephron segments under pathophysiological conditions (Figure 1). This approach allowed these investigators to do the following: (i) study cellular ATP dynamics both in the renal cortex (including the glomeruli) and in the AKI-relevant corticomedullary tissue regions; (ii) use toxic metabolic inhibitors of OXPHOS and glycolysis; and (iii) test multiple experimental conditions form the same kidney for the first time.[6] None of these advantages and features could have been realized using previous approaches. Important, new results confirm that renal tubular cells' energy dynamics using this culture system recapitulate the changes *in vivo*, and that PT tubule segments were strongly dependent on OXPHOS, in contrast to DT, whereas podocytes relied on both OXPHOS and glycolysis.[6] The results showing a high level of ATP generation in podocytes are consistent with the recent visual demonstration of the rapid and substantial metabolic sensing and responsiveness of podocytes in the intact kidney *in vivo*.[7]

To demonstrate the utility of their new model for studying mechanistic aspects of renal pathologies, the investigators applied their system in 2 kidney-injury models. In a model of AKI, this new experimental approach demonstrated that PTs (including corticomedullary PTs) have high sensitivity, that DTs are resistant to ischemic injury, and that the longer ischemic time resulted in poorer ATP recovery in PTs.[6] In addition, the application of a model of cisplatin nephropathy found that ATP in PTs decreased first, followed by a decrease in DTs, and pharmacologic inhibition of cisplatin uptake improved ATP recovery in PTs.[6] Finally, this study demonstrated the potential use of the novel ATP imaging system in drug development. Studying the mode of action of a mitochondria protection reagent in

the cisplatin nephropathy model, the investigators found diminished cellular ATP depletion in PTs.[6] These studies convincingly demonstrated that this new ATP biosensor kidney slice–culture system is a powerful tool for evaluating the nephrotoxicity of new drugs and promoting the research and discovery of drugs that improve cellular energy dynamics.

This new model and study have some limitations. As no blood perfusion occurred in this tissue-slice approach, the applied AKI model was a period of anoxia, rather than the commonly used ischemia–reperfusion injury. The results need to be interpreted in accordance with the lack of hemodynamic factors and other AKI pathogenic components in this model. In addition, the weak ATP biosensor signals do not allow measurements to be performed in some renal cell types, including mesangial and endothelial cells. The cellular heterogeneity of baseline GO-ATeam2 signals may be due to different chicken beta-actin promoter (CAG promoter) activities driving varying fluorescence resonance energy transfer–probe expression levels in different renal cell types. Alternatively, the high level of expression observed in podocytes, thick ascending limb–distal tubule, and collecting ducts may be the result of the generally high rate of protein synthesis in these cells.[8]

Future MPM imaging applications of this powerful ATP biosensor model are expected to shed light on further important details of renal cell energy dynamics, including in previously overlooked, but key, regulatory cell types, such as the macula densa.[9] This technical advance is a highly valuable new element of the kidney-research toolbox for mechanistically investigating alterations in cell metabolism, and kidney disease pathobiology, and for studying the effects of drug treatments on energy sources in different kidney cell types.

ACKNOWLEDGMENTS

JP-P is supported by National Institutes of Health grants DK064324, DK123564, and DK135290, and GG is supported by a Carl W. Gottschalk Research Scholar Grant from the American Society of Nephrology.

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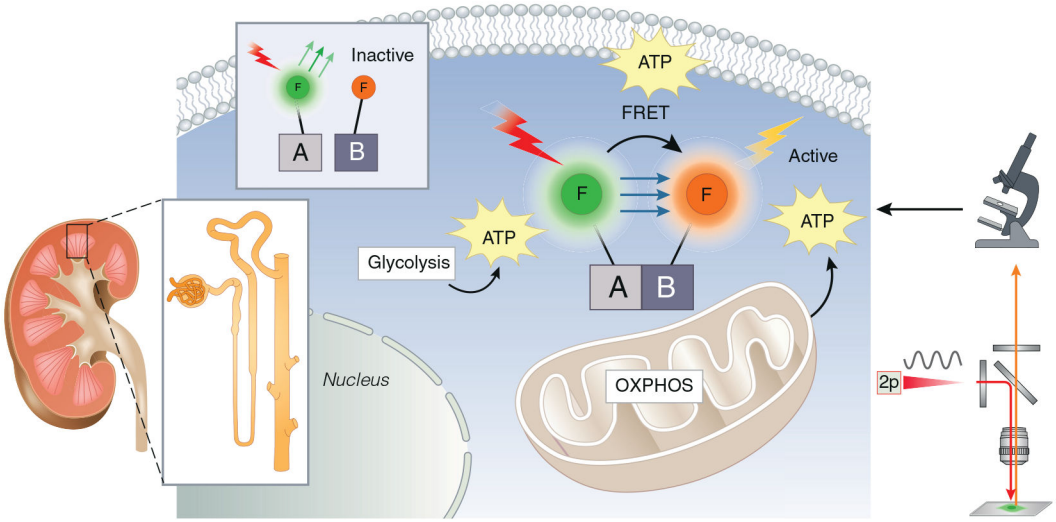

Figure 1 |. Schematic illustration of the novel kidney slice culture system–based adenosine triphosphate (ATP) biosensor as a technical advance for studying ATP dynamics in different renal cell types.

The ATP-induced fluorescence resonance energy transfer (FRET) between the GO-ATeam2 fluorescent protein pairs (illustrated by A and B, and green and orange fluorescence [F]) is illuminated and quantified by 2-photon (multiphoton) microscopy over time, as a readout of cellular ATP dynamics. The main intracellular sources of ATP synthesis, mitochondrial oxidative phosphorylation (OXPHOS), and cytosolic ATP production from glycolysis are indicated.