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Commentary

Intracellular ATP Levels: Challenge to the Current Consensus and Its Implications for Intracellular Signaling

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Abstract: Work with novel indicators that report intracellular ATP concentrations with improved spatial and temporal resolution have challenged the current consensus that under physiological conditions, intracellular ATP concentrations are not rate-limiting to enzymatic reactions. Recent data from cardiac myocytes and cultured neurons show marked fluctuations of intracellular ATP levels, as well as evidence for compartmentalization. It is likely that the availability of these genetically encoded indicators will produce rapid progress in the mapping of the dynamics of intracellular ATP concentrations in various types of cells. Here, a brief account of the most recent indicators is provided as well as a review of how natural evolution appears to have obviated the potential shortage of the ATP supply to one of key enzymes of the cyclic AMP signaling cascade, adenylyl cyclase 9.

Keywords: ATP; cAMP; biosensors; protein phosphorylation; myocardium; oxidative burst; proteasome; neurotransmitter release

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A recent break-through study [1] utilized a novel single-wavelength genetically-encoded fluorescent indicator [2] for measuring the intracellular concentration of ATP in cultured mouse cardiomyocytes. The results upend the previously held view that ATP concentrations in the heart are in the region of 4–8 mM [3,4]. Temporal variation estimated to be between 0.3–1 mM of intracellular ATP concentration during the heart cycle was shown. Moreover, evidence was provided that ATP sensitive K^+-channels become active under low ATP conditions in diastole, which was previously thought to be impossible due to the high levels of ATP [3]. The sensor developed by Lobas et al. has been further improved to enhance its dynamic range and deployed to report ATP levels in synaptic boutons of cultured hippocampal neurons [5]. The results show heterogeneity of the initial concentrations of ATP between individual boutons and a marked as well as more uniform depletion ($\geq$50%) of intracellular ATP levels during the firing of action potentials. Limitations, e.g., sensitivity of the ATP biosensors to changes of intracellular pH [2,5] notwithstanding, these technologies have the potential to fundamentally change our understanding of ATP availability in cells with rapid fluctations of energy demand under physiological conditions. In addition to myocardiocytes and neurosecretory cells [5], skeletal muscle contraction during exercise and the respiratory burst of neutrophil granulocytes and monocytes [6,7] spring to mind. Furthermore, the spatial variation of ATP levels will likely prove to be significant [2,5]. Thus issues such as the efficacy of ATP-site inhibitors of enzymes, most notably protein kinases [8], may need to be revisited even in cells not showing paroxysms of metabolic activity. Towards the pathological domain, the sensitivity of the ubiquitin-proteasome system to intracellular ATP concentrations, which follows an optimum curve [9], appears significant. As per pathological settings, variations of intracellular ATP levels in breast cancer cells reportedly have a significant impact on malignancy [10].

Sympathetic stimulation of heart function requires the intracellular signaling molecule cyclic AMP. Cyclic AMP is produced from ATP upon the activation of beta-adrenergic receptors at the cell surface. In considering which signalling pathways in the heart could

be affected by low levels of ATP, Rhana et al. [1] flagged the peculiar ATP-dependence of adenylyl cyclase 9 (AC9), one of the enzymes that generate cyclic AMP in the heart. The regulation of AC9 is unusual [11]. When in complex with the receptor-activated stimulatory protein Gsα, the enzyme shows marked auto-inhibition, which is mediated by a short, linear auto-regulatory motif in its isoform-specific carboxyl-terminal domain [12,13]. In the presence of activated Gsα, the auto-regulatory motif docks into the substrate binding-site of the enzyme causing a 40-fold reduction in the affinity for its substrate, ATP [13,14]. Importantly, in membranes prepared from rodent heart, the predominant form of AC9 is truncated; the carboxyl terminal domain, which contains the auto-regulatory motif is undetectable, likely cleaved proteolytically [12,15]. A similarly shortened AC9 protein is found in human heart samples [12]. While the exact mode of cleavage is yet to be elucidated, it is clear that the potential for diminished cyclase activity due to reduced concentrations of ATP is largely eliminated in rodent as well as human heart.

A second, radically different solution for the ATP to AC9 supply problem developed in bony fish. Species that appeared after teleost-specific genome duplication (TGD) have two AC9 genes. The respective primary sequences of the proteins encoded by these genes are closely similar to that of the single mammalian orthologue. However, only one of the teleost AC9 paralogues harbors the carboxyl-terminal auto-regulatory motif, which is highly conserved from hagfish to human (Figure 1). The RNA-seq expression tables in the PhyloFish database [16] show that in all the post-TGD species tested, the heart only expresses the gene for the AC9 paralogue that lacks the auto-regulatory motif. Thus, subfunctionalization of the AC9 genes after TGD has obviated the potential danger of suboptimal cardiac AC9 activity due to low ATP sensitivity induced by auto-inhibition. In zebrafish, knock-down of the non-autoinhibited AC9 gene caused severe acute heart failure within three days post fertilization, indicating an essential role of the enzyme in cardiac function [17].

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human          IRVQVDGSIGRSPTDE
mouse          IRVQVDGSIGRSPTDE
hagfish        YHAQVDGSIGRSPADE
sea lamprey    IHTQVDGSIGRSPAEE
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Figure 1. The auto-regulatory motif of AC9 is unique to vertebrates species and highly conserved. The single amino acid codes of the primary sequences of the auto-regulatory motif in the isoform-specfic carboxyl-teminal domain of AC9 are shown. In human AC9 these correspond to positions 1263–1278 (data from Genbank and Ensembl databases).

Taken together, ensuring the operation of AC9 in the heart independently of the fluctuations of intracellular ATP levels appears to be a significant beneficial trait during natural evolution that has produced at least two distinct solutions: proteolytic cleavage in mammalian heart and an ohnolog AC9 gene in post-TGD bony fish. A third, as yet only partly tested, possibility is dynamic modulation by protein phosphorylation [4]. As per PhosphositePlus (https://www.phosphosite.org/ (accessed on 29 October 2024)), both serines in the autoregulatory motif can be phosphorylated, and mutation of S1273 to alanine reduced the efficacy of autoinhibition [4].

One remaining question is: why has auto-inhibition of AC9 uniquely developed in vertebrates in the first place? A potential mechanistic answer is suggested by recent work by von Zastrow et al. They have shown that upon activation of Gs-coupled receptors, AC9 from among AC1, AC3, and AC5 is selectively internalized through an endosomal pathway in HEK239 cells [18] as well as medium spiny striatal neurons in culture [19]. Moreover, they found that the internalized AC9_Gsα complex is transported to the vicinity of the cell nucleus and can activate downstream cAMP-sensitive mediators. It is tempting to speculate that the powerful autoinhibition of AC9 in the presence of active Gsα serves to target activated cAMP production selectively, e.g., to a cellular compartment that can

remove the occlusion of the ATP substrate site by changing the state of phosphorylation of the autoregulatory motif. Autoinhibition ensures that only a minimal amount of cAMP is "spilled" in the cytoplasm during the transit of AC9_Gsα from the plasma membrane to the site of delivery.

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