Learning the ABCs of ATP release

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Andrew E. Libby[‡], Bryce Jones[§], and Moshe Levi[‡,1]

From the [‡]Department of Biochemistry and Molecular and Cellular Biology and the [§]Pharmacology and Physiology, Georgetown University, Washington, D. C. 20007

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ATP plays important roles outside the cell, but the mechanism by which it is arrives in the extracellular environment is not clear. Dunn *et al.* now show that decreases in cellular cholesterol levels mediated by the ABCG1 transporter increase ATP release by volume-regulated anion channels under hypotonic conditions. Importantly, these results may imply that cells that handle cholesterol differently might experience differential extracellular ATP release during hypotonicity.

ATP is well-known as the energy currency of the cell, but it can also be exported to act as a signaling molecule. Extracellular ATP can bind to cell surface purinergic receptors, including the ion channel P2X and the G protein–coupled receptor P2Y, affecting processes such as inflammation, tumor-host interactions, apoptosis, vascular barrier function, and neuronal function (1). Despite the diverse and important roles for extracellular ATP signaling that have been described over the last 60 years, the exact mechanisms by which ATP is exported have remained elusive. Dunn *et al.* now take a major step forward in this field, reporting an unexpected link between cellular cholesterol levels and ATP release made possible by a gene collection 11 years in the making (2).

How is it possible that our understanding of ATP release lags so far behind our knowledge of its functional mechanisms? First, the complexity of these systems has hindered detailed studies. A key step forward in this regard was the discovery that cultured cells can release ATP in response to cellular swelling under hypotonic conditions, thus providing an ideal *in vitro* system to study ATP export (3). Thanks in part to this approach, it is currently thought that release of ATP may involve both ATP pores and fusion of ATP-containing vesicles at the plasma membrane.

In particular, previous loss-of-function studies identified leucine-rich repeat–containing protein 8A (LRRC8) as involved in the export of ATP induced by hypotonic conditions (4). LRRC8 is a functionally critical component of VRAC,[2] which plays an important role in maintaining cellular volume under hypotonic extracellular conditions by permitting Cl^- and organic solute efflux, providing a viable explanation for ATP export (5). But how is VRAC-mediated ATP-release triggered? The loss-of-function approach has not provided more answers here, perhaps due to functional redundancy of the biomolecules involved.

Dunn *et al.* tackle this problem by taking an opposite approach: using a gain of function screen of the most extensive ORF library to date in combination with efficient *in vitro* assays. Their library contained more than 17,000 ORFs, or 90% of nonredundant protein-encoding genes from humans, enabling nearly exhaustive screening for mediators of ATP export. Importantly, the authors also found a way to simplify readouts compared with monitoring exported ATP levels with traditional luciferin-luciferase bioluminescence assays. Because P2Y receptors indirectly activate calcium release from the endoplasmic reticulum (6), the authors were able to read out VRAC-dependent increases in extracellular ATP using the FLIPR Tetra technology to detect calcium levels. After screening, the authors identified cells overexpressing two transcript variants of ABC subfamily G member 1 (ABCG1) as having higher calcium responses, leading to a proposal that ABCG1 mediates ATP export from cultured cells in a VRAC-dependent manner (Fig. 1). The authors validated this proposal with extensive controls, such as knocking down LRRC8A in ABCG1-overexpressing cells, adding an extracellular calcium chelator, and testing other stimulants and inhibitors. They also showed that the ATPase activity of ABCG1 is required for this effect by overexpressing a catalytically defective mutant. The existence of the two variants explains why ABCG1 would not have been found in a loss-of-function screen, highlighting the complementarity of this approach.

ABCG1's identification in ATP release is unexpected because the transporter is much better known as a cholesterol exporter, decreasing cellular cholesterol levels (7). Surprisingly, when the authors retested 39 other ABC transporters in their assay, they found that only cholesterol-exporting members of the ABC family (ABCG1, ABCG4, and ABCA1) could evoke a hypotonicity-induced calcium release in overexpressing cells, leading the authors to speculate that hypotonic-induced ATP export could be achieved simply by modulating cellular cholesterol levels directly. Accordingly, the authors nicely demonstrated that ATP export and intracellular calcium release could be achieved by using methyl-β-cyclodextrin (MβCD) to deplete cellular cholesterol levels. This effect was attenuated using VRAC inhibitors, indicating that the cholesterol-modulated hypotonicity-evoked effects were dependent on the VRAC

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[1] To whom correspondence should be addressed. E-mail: moshe.levi@georgetown.edu.

[2] The abbreviations used are: VRAC, volume-regulated anion channel; ABC, ATP-binding cassette; MβCD, methyl-β-cyclodextrin.

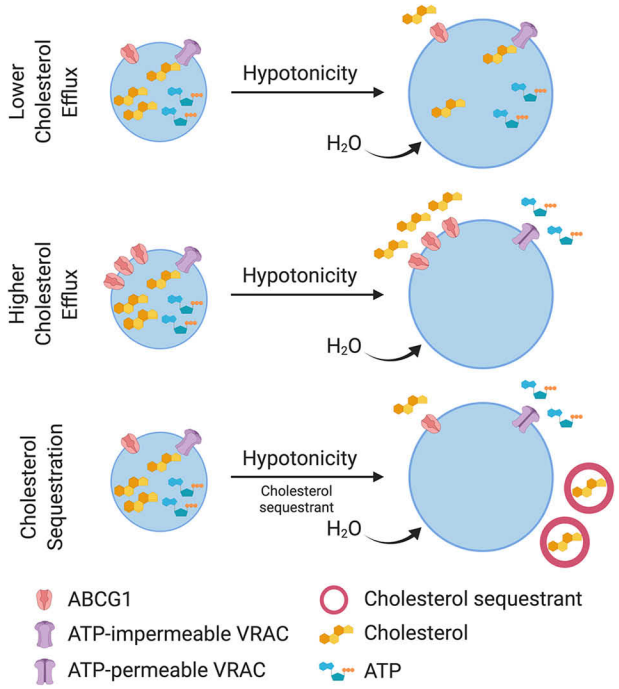

Figure 1. Proposed model for hypotonicity-induced ATP release. At lower levels of ABCG1 expression (*top*), hypotonicity does not induce VRAC-dependent ATP release, possibly due to excess cholesterol acting as a channel blocker. When ABCG1 expression is increased (*middle*), ABCG1-dependent cholesterol efflux stimulates VRAC-dependent ATP release under hypotonic conditions. When cholesterol is pharmacologically sequestered (*bottom*), hypotonicity induces VRAC-dependent ATP release. This figure was created with BioRender.

complex. Finally, the authors demonstrated their findings in a primary neuron culture system. Using cultured cerebellar granular neurons from mice, the authors show that depletion of cholesterol using MβCD mediates ATP release in response to hypotonic conditions in a VRAC-dependent manner, and they also demonstrate an attenuation of hypotonic ATP release by supplementing the cells with excess cholesterol.

The study by Dunn *et al.* raises a variety of interesting questions. For example, the authors show that ATP release in response to hypotonicity can be achieved simply by modulating ABCG1 and/or cholesterol levels, but what is the direct mechanism by which a reduction in cholesterol mechanistically achieves ATP release? The authors postulate that cholesterol may block the LRRC8A complex to prohibit ATP release under conditions where cholesterol is abundant. When cholesterol is depleted (either artificially or by overexpressing ABCG1), this would cause the LRRC8A pore to become unblocked. Such a mechanism will need to be confirmed in the future. This might be achieved by testing for cholesterol binding and by mutating residues of the LRRC8A-VRAC complex that putatively bind cholesterol. Furthermore, the study raises the question of whether intracellular proteins that bind to and effectively lower available cholesterol concentrations might also have an effect on VRAC-mediated ATP export.

Next, the authors note that several other molecules have been identified as possibly mediating ATP release, such as the pannexin/connexin channels and the SLCO2A transporter. For example, adipocytes can release ATP by a pannexin-1–mediated mechanism, which is modulated by insulin and glucose (8). Is ABCG1 or cholesterol involved in this process as well, or do other regulators await discovery? Equally important, the study also raises the question of whether cholesterol-altering diseases may impart previously unrecognized effects on extracellular ATP release and signaling. Diseases such as fatty liver, atherosclerosis, and familial hypercholesterolemia lead to altered cellular cholesterol levels, and determining whether extracellular ATP release or signaling are altered in these conditions would be valuable to improving our understanding and potentially therapeutic approaches toward these maladies. Finally, the results of this study might imply that cells that handle cholesterol differently might experience differential extracellular ATP release. For example, cells that store and sequester large amounts of cholesteryl-esters within intracellular lipid droplets might be more sensitive to ABCG1-mediated cholesterol depletion and subsequent ATP release compared with cells that do not exhibit extensive intracellular cholesterol sequestration via lipid droplets.

By identifying ABCG1 and its substrate cholesterol as responsible for enhancing VRAC-mediated ATP release, Dunn *et al.* have provided building blocks for further examination as fundamental as our own ABCs. We hope the results of this study will bring together talented scientists from both the lipid and ion transporter fields to elucidate complex functionality of systems that would otherwise go unstudied.

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