reduction in MR $S^{843P}$ compared with wild-type, despite hyperkalemia which normally elevates the level of phosphorylation. Very high levels of administered aldosterone (>10-fold those in sodium deficiency) elevated pendrin and BI $H^+$ ATPase (MR-induced proteins) 2- to 3-fold; equivalent doses in wild-type mice produced little or no effect. This is altogether unsurprising, given that the majority of MR $S^{843}$ were nonphosphorylated in the transgenic mice; wild-type mice on regular mouse chow have much higher MR $S^{843P}$ (and therefore much lower levels of activable MR $S^{843}$) than the mutants.

I have no doubt that dephosphorylated MR S843 in intercalated cells can be activated by aldosterone. The authors show equivalent activation by cortisol; without any "protective" mechanism like 11βHSD, the default agonist in vivo is therefore cortisol (corticosterone in the mouse). This is not merely a default position, but clearly supported by the different phenotypes of the MR knockout (MRKO) and the aldosterone synthase null ($AS^{-/-}$) mouse (Funder, 2006). MR are clearly essential for $Na^+$ homeostasis: on a low salt intake, MRKO mice perish. Aldosterone is not essential; in $AS^{-/-}$ mice on a low salt intake, their blood pressure falls slightly, their renin-angiotensin system is markedly activated, they become modestly hyperkalemic, but (in the presence of glucocorticoids) they survive (Makhanova et al., 2006). Occam's razor would say that they are doing this via their intercalated cells, with the very elevated angiotensin overcoming the modest hyperkalemia in terms of dephosphorylation of intercalated cell MR $S^{843P}$, and endogenous glucocorticoid then allowing sufficient Na-Cl reabsorption to maintain life.

The authors' yoking the discovery of a single phosphorylation site in the MR ligand binding domain to solving a physiological (and inevitably, clinical) conundrum is a masterstroke of translational research. By showing the opposite effects of angiotensin and hyperkalemia on MR $S^{843}$ phosphorylation, the authors have elegantly demonstrated how these two different stimuli can act via MR to maintain electrolyte homeostasis. Given the absence of 11βHSD2 from intercalated cells, the ligand for dephosphorylated MR $S^{843}$ is presumptively cortisol rather than aldosterone. If this is the case, the authors have solved not one but two enigmas. The first is the MR-mediated differential response to volume depletion and potassium loading; the second is the different response to sodium restriction in the MRKO and $AS^{-/-}$ mouse.

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# Cardiolipin and the Nlrp3 Inflammasome

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**The Nlrp3 inflammasome is a key controller of the proinflammatory cytokine IL-1β. Iyer and colleagues (2013) demonstrate that Nlrp3 activators promote an interaction between cardiolipin in mitochondria and Nlrp3. Cardiolipin is thus a hydrophobic danger signal that, upon translocation to the outer mitochondrial membrane, activates Nlrp3, promoting inflammation.**

The Nlrp3 inflammasome is a multiprotein complex that regulates caspase-1, the protease that processes pro-IL-1β into its mature form. Given the importance of IL-1β as a proinflammatory cytokine, there has been interest in understanding the regulation of Nlrp3. Studies in Nlrp3-deficient mice as well as humans all point to Nlrp3 as an important driver of inflammatory diseases as diverse as type 2 diabetes, Alzheimer's disease, and gout (Wen et al., 2012). Nlrp3 can be activated by a wide range of stimuli, including particles such as uric acid, islet-associated polypeptide, β-amyloid and silica, as well as factors such as ATP acting via the P2X7 receptor. This diversity of stimuli has presented a challenge concerning the mechanism of activation. A common feature appears to be the generation of reactive oxygen species (ROS) by mitochondria (Zhou et al., 2011), although how ROS actually impacts Nlrp3 is not known. In a recent paper in *Immunity*, Iyer and colleagues provide a new piece in the puzzle of Nlrp3 activation, identifying the mitochondrial phospholipid cardiolipin as an activator of Nlrp3, operating downstream of ROS (Iyer et al., 2013). Of interest, cardiolipin acts as a classic danger signal, given that it appears on the outer membrane of mitochondria only when the mitochondria become dysfunctional.

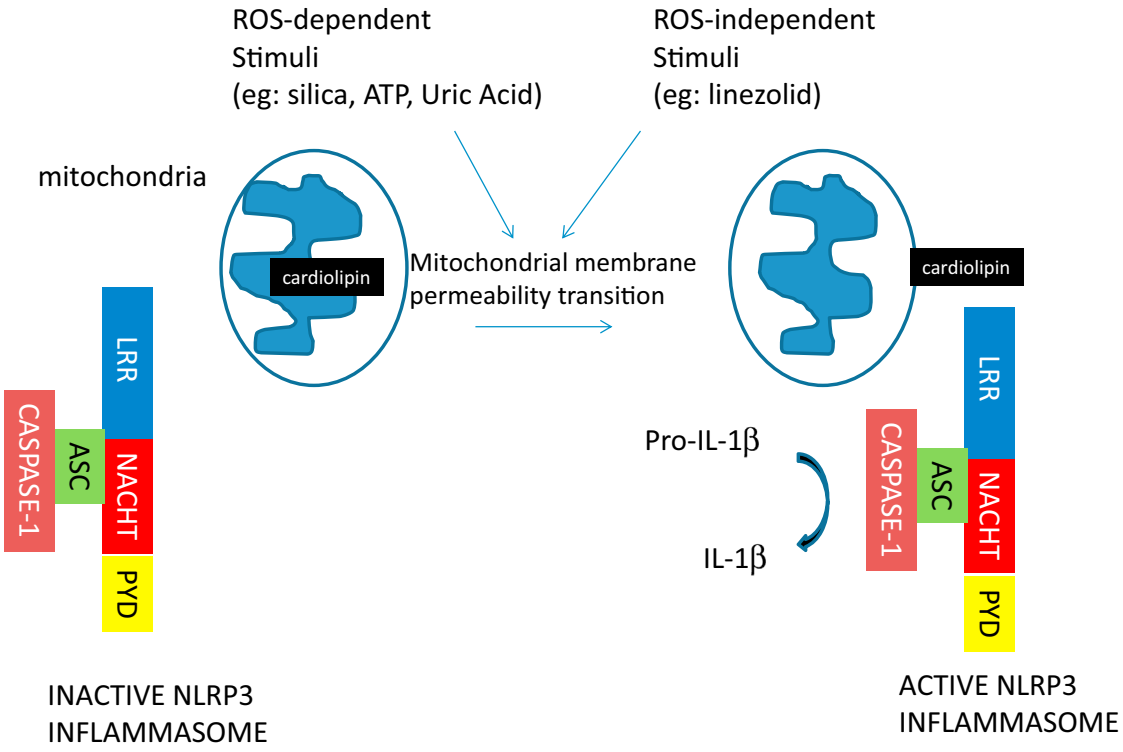


**Figure 1. Cardiolipin Activates the Nlrp3 Inflammasome**
Both ROS-dependent and -independent activators of Nlrp3 cause cardiolipin to translocate from the inner to the outer mitochondrial membrane. Cardiolipin then binds to the LRR domain of Nlrp3, explaining why Nlrp3 localizes to the mitochondria. This interaction leads to activation of the Nlrp3 inflammasome, promoting caspase-1-mediated cleavage of pro-IL-1β to mature IL-1β.

The study began with an observation regarding the antibiotic linezolid, which causes myelosuppression—suppression of erythroid progenitors in bone marrow—as a side effect. The authors wondered if the myelosuppression was due to a proinflammatory effect induced by linezolid, since the inflammatory process is known to affect the numbers of erythroid and myeloid precursors. They found no induction of inflammatory cytokines such as TNF or IL-6 in macrophages. However, assaying for IL-1β production, they observed a strong increase in the levels of mature IL-1β, an effect not observed with other antibiotics. The effect on IL-1β was Nlrp3 dependent since it was attenuated in Nlrp3-deficient mice and in mice lacking the inflammasome platform protein Asc. Linezolid also increased neutrophil infiltration into the peritoneum, which was also Nlrp3 dependent. In the final in vivo experiment, the authors found that the effect of linezolid on myelopoiesis required Nlrp3, providing a mechanistic basis for this side effect of linezolid.

The authors then explored the mechanism by which linezolid activates Nlrp3. As with other Nlrp3 activators, activation by linezolid required an efflux in potassium. However, antioxidants such as the mitochondrially targeted antioxidant mito-TEMPO had no effect on the response, which is of substantial interest given that all other Nlrp3 activators are ROS dependent. Consistent with previous studies showing that linezolid causes mitochondrial dysfunction, the authors observed a decrease in $NAD^+$, ATP, and cytochrome oxidase activity. Indeed, Cyclosporine A, which prevents the mitochondrial membrane permeability transition, inhibited the response to linezolid, implicating the membrane permeability transition in the response.

Since Nlrp3 is known to associate with mitochondria, the authors tested which mitochondrial component might bind to it. To test lipids, they immunoprecipitated Nlrp3 from $^{14}$C-linoleic acid-labeled cells activated with ATP or silica following LPS priming (a standard method to activate Nlrp3 and induce production of pro-IL-1β), and observed a major increase in cardiolipin binding to Nlrp3. This lipid is usually found in the inner mitochondrial membrane and has a unique structure among phospholipids, containing two diacylated phosphatidyl groups joined by a glycerol bridge. Using a protein-lipid overlay assay, the authors found that cardiolipin binds to the leucine rich repeat (LRR) region of Nlrp3. To test the role of cardiolipin in Nlrp3 activation, the authors used two different approaches to decrease cardiolipin levels in cells: (1) incubation with palmitate in serum-free medium or (2) knockdown of cardiolipin synthase. Both of these approaches interfere with cardiolipin biosynthesis. Under both scenarios, activation of Nlrp3 was impaired, as was Nlrp3 binding to mitochondria. In fact, activation was impaired with all stimuli tested, both those that act via ROS, and linezolid, which does not, indicating that cardiolipin is essential for Nlrp3 activation. Cardiolipin has been shown to be involved in apoptosis, regulating caspase-8. This role in apoptosis is separate from its

effect on Nlrp3, however, since apoptotic activators such as staurosporine do not activate Nlrp3.

This study is important for a number of reasons. First, the results show that it is possible to activate Nlrp3 in a ROS-independent manner, raising the question as to what other stimuli, more physiological than linezolid, might achieve this effect. It appears likely that any mitochondrial disruptor that causes cardiolipin to move to the outer mitochondrial membrane will activate Nlrp3. Second, the study shows that cardiolipin is a common regulator of Nlrp3, whether ROS is involved or not. Cardiolipin may therefore be a key downstream player in the effect of ROS on Nlrp3, although how ROS might cause cardiolipin translocation needs to be explored. The change in the mitochondrial membrane permeability transition may be involved in this translocation. Third, cardiolipin binds to the LRR of Nlrp3, but the consequences of this interaction are unclear. Is there a conformational change that allows caspase-1 activation in the Nlrp3 inflammasome complex? Or, is localization to the mitochondria sufficient to trigger activation? One complication is that in cells containing an active inflammasome, large specks containing Asc and Nlrp3 form, but not proximal to mitochondria. The role of mitochondria for the functioning of these specks needs to be addressed. Finally, the study suggests that cardiolipin fulfils the criteria of a hydrophobic danger signal, as first proposed by Seong and Matzinger (2004). Such a signal would be exposed in response to stress that triggers mitochondrial dysfunction. The study from Iyer et al. (2013) suggests that inflammatory insults caused by infection or noxious agents such as silica cause cardiolipin to move from the haven of the inner mitochondrial membrane to the cytosol-facing outer membrane, allowing Nlrp3 recruitment and activation and leading to the production of the important proinflammatory cytokine IL-1β, as shown in Figure 1.

The mitochondrion, therefore, yet again takes center stage as an organelle that provides a readout for the state of the cell, in this case via cardiolipin translocation (Tschopp, 2011). The study therefore brings us a step closer to understanding the complex regulation of the Nlrp3 inflammasome, a fascinating multiprotein complex that is essential for health and yet must be kept under tight control to prevent disease.

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# BDNF (*l*)*rising* from Exercise

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**Exercise produces many beneficial effects on brain health, in part by increasing hippocampal BDNF levels; however, the mechanism underlying this BDNF regulation remains unknown. In this issue of *Cell Metabolism*, Wrann et al. (2013) show that exercise induces hippocampal *Bdnf* gene expression by stimulating expression of FNDC5, the precursor of irisin, via the transcriptional complex PGC-1α/Errα.**

Exercise, especially endurance exercise, offers many health benefits to the human brain, including improving cognitive function, reducing risk for Alzheimer's disease, and alleviating depression (Cotman et al., 2007; Mattson, 2012). These beneficial effects implicate the hippocampus as a major target of exercise, as the hippocampus is crucial for cognitive function and is one of the brain regions severely affected in Alzheimer's disease and depression (Duman and Aghajanian, 2012; Huang and Mucke, 2012). Indeed, exercise increases adult neurogenesis in the dentate gyrus (DG, part of the hippocampus), dendritic complexity of DG granule neurons, and synaptic plasticity (the cellular basis of learning) in the pathway connecting the entorhinal cortex to the DG (Eadie et al., 2005; Farmer et al., 2004). Substantial evidence indicates that exercise affects the hippocampus largely by inducing expression of brain-derived neurotrophic factor (BDNF) (Mattson, 2012). BDNF is a potent regulator of neuronal survival, adult neurogenesis, synaptogenesis, and synaptic plasticity (Park and Poo, 2013). However, the biochemical pathway that is activated by exercise and induces *Bdnf* gene expression remains unknown. Wrann et al. (2013) now report identification of a novel biochemical pathway linking an exercise-induced, secreted factor from skeletal muscle to hippocampal *Bdnf* gene expression (Figure 1).

Spiegelman and colleagues have recently discovered that exercise induces expression of FNDC5, a type I membrane