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A central role for mitochondrial-derived vesicles in the innate immune response: implications for Parkinson's disease

Thomas A. Ryan*, **David A. Tumbarello***

Mitochondria are well known for their function in energy production; however they also play a crucial role in phospholipid transfer, inflammation, calcium balance and cell death, positioning them as a central regulator of cellular homeostasis. The cell therefore relies on quality control mechanisms to limit mitochondrial damage and the production of harmful reactive oxygen species (ROS). To date, numerous mitochondrial quality control (mitoQC) pathways have been defined, with mitophagy, the autophagic degradation of entire mitochondria, being the most extensively studied. Locally directed repair pathways also exist and we have recently elucidated key mechanisms of one of these mitochondrial stress response pathways regulated by the endosomal adaptor – Toll-interacting protein (Tollip) (Ryan et al., 2020). In this pathway, damaged mitochondrial proteins and lipids are selectively transported as mitochondrial-derived vesicles (MDVs) into the endolysosomal system to facilitate cargo degradation. However, these MDVs are heterogeneous, with each discrete cargo trafficked via a specific route and destination. Since their initial identification demonstrating the segregation of the outer membrane mitochondrial-anchored protein ligase and its subsequent shuttling to peroxisomes (Neuspiel et al., 2008), MDVs have been shown to incorporate a host of different proteins from the inner and outer membranes or matrix. Indeed, it appears their cargo and potentially their membrane composition defines the trafficking machinery required, the particular route taken and their destination.

In addition to MDVs providing cargo-specific routes of degradation and recycling, their involvement in regulating innate immune pathways has also been identified (Matheoud et al., 2016). Tollip was originally identified as a regulator of the innate immune response through its suppression of interleukin-1 signaling (Burns et al., 2000). Our recent findings demonstrate that Tollip, in coordination with Parkin, facilitates the entry of MDVs carrying the outer membrane protein TOM20 into the endosomal system to enable their transport to the lysosome (Ryan et al., 2020). This pathway is upregulated in response to mitochondrial stress, suggesting it may have a significant role in maintaining the health of the mitochondrial network by limiting cell death often associated with diseases such as Parkinson's disease. Tollip has been shown to be protective in Huntington's disease by mediating the clearance of huntingtin polyQ aggregates (Lu et al., 2014). Tollip also interacts with and stabilizes the innate immune regulator, Stimulator of Interferon Genes (STING), by preventing its lysosomal degradation (Pokatayev et al., 2020). Interestingly, the presence of polyQ protein aggregates sequesters Tollip from STING, thus dampening the innate immune response (**Figure 1**).

The loss of PTEN induced putative kinase 1 (PINK1) and Parkin function in a chronic *in vivo* model of mitochondrial dysfunction potentiates the innate immune response and neurodegeneration, which can be reversed by blocking the STING-mediated type I interferon response (Sliter et al., 2018). This is in addition to the role of PINK1 in positively regulating interleukin-1β signaling by interacting with Tollip leading to its dissociation from interleukin-1 receptor-associated kinase 1 (Lee and Chung, 2012). These data are interesting as they align with a model in which the innate immune response and the mitochondrial stress response are intricately linked. Moreover, it has been suggested that PINK1 and Parkin function to suppress MDV formation and subsequently lysosomal-dependent mitochondrial antigen presentation (MitAP) (Matheoud et al., 2016). Importantly, the presentation of the mitochondrial enzyme OGDH on MHC class I molecules required MDVs and utilized Snx9, Rab7 and Rab9 compartments at different stages along their route to the cell surface. Building on these data, a very recent study has shown that the trafficking of mitochondrial constituents into extracellular vesicles (EVs) is also dependent on Snx9, whilst Parkin-dependent routing into MDVs suppressed this pathway and subsequently prevented inflammation (Todkar et al., 2020). These findings are made more intriguing by the fact that MHC class I presentation has already been observed in dopaminergic neurons. In this context, it would also be of great interest to determine if Tollip participated in MitAP through its regulation of a subclass of MDVs. What does however appear apparent is that induction of severe mitochondrial stress would initiate Parkin-dependent responses, such as mitophagy, limiting its ability to inhibit MitAP. Thus, Parkin sequestration to regions of mitochondrial damage may result in the loss of its capacity to modulate the immune response. How this relates to more focused local oxidative mitochondrial damage and the potential impact of Tollip-Parkin MDV cargo sequestration away from inflammatory EVs is currently unknown.

These studies suggest a delicate balance is required to maintain the various Tollip-dependent pathways and perturbation of any one of these could have knock-on effects that lead to a dysregulated response. In the context of Parkinson's disease, elevated mitochondrial stress may lead to a saturation of Tollip function along the MDV route, while Tollip's role in stabilizing STING would be compromised. Moreover, Parkin would potentially also be sequestered to alleviate mitochondrial stress, potentially removing its capacity to regulate STING as well as MitAP (**Figure 1**). Therefore, determining the thresholds and interactions that govern the interplay of these pathways may provide important insight into therapeutic-design across several disease models.

These observations collectively demonstrate the requirement for inter-organelle communication and the recruitment of cargo adaptor proteins to fulfil the dynamic regulation and complex interrelationship of these often distinct pathways. It is already clear that at least some MDVs interact with endosomal compartments on route to the lysosome (Ryan et al., 2020), likely requiring enzymatic processing of MDV phospholipids by phospholipases as well as phosphoinositide kinases and/or phosphatases. It is also well established that mitochondria have an intimate relationship with the endoplasmic reticulum (ER), regulating processes such as phospholipid transfer, mitochondrial fission, and intracellular signaling. However, an area that is relatively unexplored in MDV biology is the role of the ER. Matheoud and colleagues (2016) hypothesize that mitochondrial peptides derived from the lysosome are processed then loaded onto MHC I molecules in the ER. Interestingly, STING is an ER-associated protein, perhaps providing spatial proximity to facilitate associations with Tollip- and PINK1-Parkin-mediated pathways during mitochondrial stress. Recently, the E3 ubiquitin ligase RNF26 has been shown to organize a newly identified regulatory complex that modulates STING-mediated innate immune signaling at the ER (Fenech et al., 2020). This is an interesting finding in light of earlier reports illustrating that Tollip associates with RNF26 via p62 to regulate endosomal positioning and cargo transport to the lysosome (Jongsma et al., 2016). It would therefore be interesting to determine whether Tollip acts as a key player linking mitochondrial-ER contacts to the innate immune response. Mitochondrial-ER contacts are extensively documented in the literature and it would certainly make sense for these inter-organelle associations to play a role in MDV biology. Indeed, ubiquitylated mitochondria can be "cradled" by the ER, which provides a platform for their targeted degradation via autophagy (Zachari et al., 2019). With respect to MDV pathways, it would be plausible for the ER to function at the earliest stages, perhaps assisting mitochondrial arrest or providing sites of mitochondrial constriction to instigate MDV budding. Then, as previously suggested, the ER may play a role in the loading of mitochondrial peptides onto MHC I molecules, while allowing MDV-dependent EVs to provide a mode to regulate the inflammatory response.

The role of Parkin in MDVs appears to vary dependent on the origins of the cargo, with it being required for the transport of TOM20 MDVs to the lysosome, but not essential for their formation or "budding off" from mitochondria (Ryan et al., 2020). In contrast, Parkin is required for the formation of PDH MDVs (McLelland et al., 2014) and it functions as an inhibitor in alternative MDV pathways associated with inflammation (Matheoud et al., 2016; Todkar et al., 2020). Therefore, what defines the role of Parkin in the MDV repair pathway is likely to be cargo- and context-dependent. The requirement for Tollip, an adaptor protein containing a ubiquitin-binding domain and multiple phosphoinositide binding motifs, in the trafficking of MDVs (Ryan et al., 2020) certainly suggests that following their "budding off," MDV translocation could be directed through adaptor-dependent association with motor proteins or to specific vesicular compartments via phospholipid binding domains. Indeed, Tom1, Tollip's primary interactor, acts as a cargo adaptor for the actin
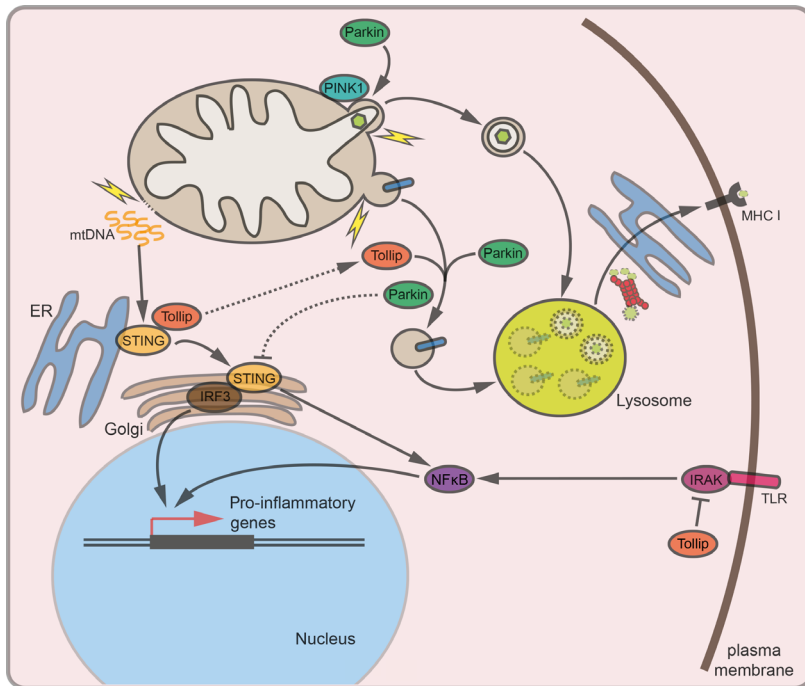
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Figure 1 │ **Mitochondrial-derived vesicles regulate multiple routes of the innate immune response.**
Following local damage of mitochondrial proteins and lipids in response to oxidative stress, mitochondrial-derived vesicles (MDVs) form and transport damaged cargo to the lysosome for degradation. These MDVs can either be single membrane vesicles derived exclusively from the outer membrane or double membrane vesicles carrying inner membrane or matrix-derived cargoes. PINK1 and Parkin are required for the formation of inner membrane-derived MDVs, while Parkin is required for the trafficking of outer membrane-derived MDVs to the lysosome. Tollip is essential for coordinating the trafficking of these Parkin-dependent outer membrane derived MDVs to the lysosome. Once in the lysosome, it is thought these broken down mitochondrial antigens can be further processed by the proteasome and loaded onto MHC I molecules in the endoplasmic reticulum (ER) prior to presentation at the plasma membrane. Damage to the mitochondria can additionally result in the release of mitochondrial DNA, stimulating the STING-mediated type I Interferon immune response. STING resides in the ER and following mitochondrial DNA activation, moves to the Golgi to activate IRF3 nuclear translocation and subsequently influences pro-inflammatory gene expression. Stimulator of Interferon Genes (STING) activation also stimulates nuclear factor κB (NFκB) mediated pro-inflammatory gene expression. The presence of Parkin and its role in mitoQC aids in the suppression of STING-mediated inflammation. In comparison, Tollip is a negative regulator of NFκB downstream of interleukin-1β (IL-1β) mediated Toll-like receptor (TLR) activation. Tollip additionally interacts and stabilizes STING expression to maintain the type I Interferon immune response. However, following mitochondrial stress, Tollip may be sequestered away from STING, resulting in a dampening of the innate immune response. IRAK: Interleukin-1 receptor associated kinase; IRF3: interferon regulatory factor 3; mtDNA: mitochondrial DNA; PINK1: PTEN induced putative kinase 1; Tollip: Toll-interacting protein.

motor Myosin VI, which has recently been implicated in regulating aspects of mitoQC (Tumbarello et al., 2012; Kruppa et al., 2018). The answer to the question of how specific MDVs traffic along discrete routes may therefore lie in their cargo-dependent recruitment and association with adaptor proteins, which in turn dictate the route and destination.

In conclusion, the multifunctional capacity of proteins that regulate the mitochondrial stress response, such as Parkin, PINK1 and Tollip, allow for their parallel and/or alternative regulation of the innate immune response (**Figure 1**). With respect to neurodegeneration, we would suggest that these carefully balanced systems operate as necessary, with their activation or inactivation being dictated by the level and/or type of stress a neuron undergoes. Immunological processes appear to be tempered in favor of stress pathways during mitochondrial dysfunction, accompanied by a switch in the functional role of certain key proteins. Indeed, the "apoptotic switch" capability of normally cytoprotective proteins like Parkin would allow for the initiation of neuronal cell death if a threshold should be reached. In Parkinson's disease, this switch may occur at a lower threshold given the apparent vulnerability of dopaminergic neurons in the substantia nigra pars compacta to mitochondrial dysfunction as a result of dopamine metabolism and α-synuclein dysfunction. Therefore, using a targeted approach to alter this threshold may provide an area for therapeutic development to augment existing protective mechanisms and further engage the immune response. The hypothesis linking neurodegenerative disorders, such as Parkinson's disease, to the innate immune system is not novel. However, it is now becoming apparent that mitoQC could be a central regulator of the immune response during neuronal stress.

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