Mitochondrial ROS generated at the complex-II matrix or intermembrane space microdomain has distinct effects on redox signalling and stress sensitivity in *C. elegans*

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Background: Excess reactive oxygen species (ROS) are implicated with numerous diseases, yet physiological ROS generation is necessary for cellular function and adaptive responses to stress. Mitochondria are a major source of ROS which can fluctuate widely in response to various environmental and cellular stimuli. The downstream responses induced by ROS may depend on the duration and rate of oxidant generation/removal as well as the subcellular microdomain in which this occurs. However, manipulating ROS levels to investigate this *in vivo* with traditional pharmacological approaches lacks precise spatial and temporal control and can have confounding effects on mitochondrial bioenergetics.

Results: We used CRISPR/Cas9 to fuse the light-sensitive ROS-generating protein, SuperNova (SN) to the C-terminus of mitochondrial complex II succinate dehydrogenase subunits B (SDHB-1::SN) and C (SDHC-1::SN) in *C. elegans*. The SDHB-1::SN and SDHC-1::SN fusion proteins localised SN to the mitochondrial matrix side of the inner membrane, and to the intermembrane space (IMS), respectively, and had no impact on complex II activity. ROS production by SN protein *in vitro* was both specific and proportional to total light irradiance in the 540-590 *nm* spectra and was unaffected by varying the buffer pH to resemble the mitochondrial matrix, IMS or the cytosolic environments. We then determined whether ROS generated at either side of the inner mitochondrial membrane with 1:1 stoichiometry has distinct effects on redox signaling, *in vivo*. Using a GFP transcriptional reporter strain, we assessed activity of SKN-1 (the *C. elegans* homologue of Nrf2), the master regulator of the antioxidant response pathway. We found that SKN-1 transcriptional activity was dependent on both the site of ROS formation and duration of ROS production: with less matrix generated ROS required for activation. In addition, there was greater phosphorylation of PMK-1, (a p38 MAPK homologue) in response to ROS generated in the matrix compared to IMS. Finally, matrix generated ROS displayed protection against subsequent exposure to simulated ischemia reperfusion injury.

Conclusions: Overall, these novel data demonstrate that the physiologic output of ROS depends on the microdomain in which it is produced. These findings may inform further studies to identify novel therapeutic strategies for diseases involving mitochondrial oxidative stress.