Selenoprotein O Protects Against Oxidative Stress and Incorporates Various Nucleotides in AMPylation

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Selenoprotein O (SelO) is a mitochondrial pseudokinase enzyme that AMPylates, or transfers an AMP molecule to, cellular proteins. However, SelO's role as an AMPylator in oxidative stress response is largely unknown. It was hypothesized that if SelO mitigates oxidative stress induced by diamide, a sulfhydryl oxidizer, and attenuates homeostatic dysfunction induced by metal deficiency by 2'2-dipridyl, then there will increased viability as compared to the knockout without SelO present. SelO conventionally utilizes ATP to conduct AMPylation. However, recent studies have shown that another known mammalian AMPylator, FicD, not only utilizes but also selectively prefers alternative nucleotides called diadenosine polyphosphates (ApnAs) for AMPylation. ApnAs, also termed alarmones, are structurally similar to ATP and are present in high concentrations during oxidative stress, signaling homeostatic imbalance. It was hypothesized that SelO can potentially utilize ApnAs in autoAMPylation. A nucleotide screening was conducted investigating Ap_3A, Ap_4A, Ap_5A, ADP, AMP, and ATP in AMPylation assays with SelO, followed by visualization with Western Blotting. Viability assays were conducted by measuring optical density (OD600) at 30-minute increments to construct a growth curve. It was found that ATP was utilized most extensively as a cosubstrate by SelO, followed by Ap_3A and ADP. Wild-type strains of E. coli with SelO presented increased viability in iron-deficient environments and under oxidative stress as compared to Knockout. These results highlight that SelO's function as an AMPylator also rescues the cell from oxidative stress and presents a potential avenue for further research into its overarching role in the cell.