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Mitochondrial Respiratory Complexes of *Ustilago maydis*

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Abstract: Respiring organisms commonly utilize a structurally and functionally conserved set of five mitochondrial respiratory protein complexes (CI to CV) that transfer electrons and pump protons to produce ATP. Despite the high conservation of respiratory complexes, there are variations in higher-order assemblies (supercomplex), accessory subunits, and regulation. For example, fungal respiratory complexes are homologous to that of mammals, but unlike those of mammals, fungi respiratory complexes function in parallel with diverse branching respiratory pathways. It is unclear whether there are structural differences between obligate and non-obligate aerobic fungi. Available structures of fungal respiratory complexes are from a single clade (Ascomycota) out of nine total clades, and high-resolution structural information for supercomplexes is currently unavailable. Here we present structures of the mitochondrial respiratory complexes in *Ustilago maydis*, an aerobic fungus in the Basidiomycota clade, all within a supercomplex assembly. We isolated functionally active respiratory complexes from *U. maydis* mitochondria and visualized them to near-atomic resolutions using single particle cryogenic electron microscopy (cryoEM). Our results help contextualize how variations in supercomplexes influence critical structural elements within complexes and may influence function. Our findings will provide additional insight into how higher-order assemblies are utilized to increase the functional plasticity of conserved mitochondrial respiratory complexes.

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