Ammonium blocks chronological lifespan extension of extreme calorie restriction in amino acid-starved yeast cells associated to Tpk1- and Tor1-dependent necrotic cell death induction

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We have previously shown that ammonium stimulates cell death in amino acid-deprived auxotrophic cells of *Saccharomyces cerevisiae* (aa-starved cells) transferred to water, whereas the same cells in absence of ammonium displayed chronological lifespan (CLS) expansion typical of extreme starvation conditions. The enhanced loss of cell viability in the presence of ammonium was accompanied by an initial small increase of apoptotic cells followed by extensive necrotic cell death. Exposure to ammonium in aqueous solution decreased CLS and induced cell death in aa-starved cells but not in nitrogen-starved cells (N-starved cells), suggesting that the ammonium effect was dependent on the inappropriate arrest of the aa-starved cells. In fact, we did not observe autophagy induction (a marker of G0 arrest) in aa-starved cells and this effect was sustained by ammonium when cells were transferred to water. However, blockage of autophagy did not significantly induce the ammonium sensitive phenotype in N-starved cells suggesting that hindrance of autophagy does not play a causative role in the shorter CLS. On the contrary, activation of PKA stimulated ammonium induced decrease of CLS in N-starved cells consistent with the observation that deletion of *MEP1*, *MEP2*, *RAS2* or *TPK1* could

partially revert the ammonium effect in aa-starved cells. Rapamycin did not alter ammonium induced decrease of CLS, consistent with PKA over-activation, however, the deletion of *TOR1* significantly rescued the ammonium effect showing that signaling through PKA pathway was dependent on the presence of *TOR1* or could be override by its absence.

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