Directed Evolution of Extreme Radioresistance

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To further our efforts to explain ionizing radiation resistance, we have taken the radiosensitive species, *Escherichia coli* strain MG1655, and subjected it to high dose ionizing radiation with the intent of generating three independent radioresistant strains that can be more easily studied than naturally radioresistant species. The protocol consisted of a series of selective cycles in which successive exponential phase cultures were exposed to increasing doses of gamma radiation. The initial dose applied killed approximately 99% of the culture. The survivors were diluted into fresh growth medium and allowed to propagate. This process of irradiation and outgrowth was repeated for 20 cycles. As the culture became more resistant to the effects of ionizing radiation the dose administered was increased. At the end of the study a purified resistant strain was recovered and its capacity to survive ionizing radiation evaluated. The evolved strains were between 1000 and 10000 - fold more resistant at 3000Gy than their parent. Complete genome re-sequencing revealed that each strain acquired its radioresistance through completely different sets of genetic changes. The analyses of these changes do not highlight a particular strategy for the acquisition of radiation resistance nor reinforce any current idea about the mechanisms underlying the radiation resistance of species such as *Deinococcus radiodurans*. Our results suggest that radioresistance is a complex phenotype requiring that function be altered in a number of proteins, and that multiple genomic solutions exist to solve this challenge. The many classes of mutations found, along with the lack of overlap in the mutations (or the mutated genes) observed from one strain to the next suggest that multiple mechanisms may contribute to radiation resistance in nature.