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Coping with the Stress of Photosynthesis, a Bacterial Response to the Reactive Oxygen Species, Singlet Oxygen ($^1O_2$)

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When photosynthetic organisms acquired the ability to produce O_2, they altered the Earth's atmosphere and the pathways that sustain life on the planet (8). Accumulation of atmospheric O_2 allowed evolution of bioenergetic pathways like aerobic respiration that conserve energy as O_2 reduction is coupled formation of a proton gradient (16). A trade-off to accumulation of atmospheric O_2 is formation of reactive oxygen species. When electrons are transferred to O_2, superoxide, hydrogen peroxide, or hydroxyl radicals are formed. These reactive oxygen species can damage biomolecules, kill cells or trigger onset of debilitating diseases, so considerable effort has gone into determining how cells sense and respond to these compounds (9, 14, 17).

In contrast, little is known about the response to another class of reactive oxygen species, singlet oxygen ($^1O_2$). $^1O_2$ is formed when energy is transferred to O_2 (4, 10), reorganizing the outer orbital electrons to generate a powerful oxidant (5) that can kill prokaryotic and eukaryotic cells. We are studying the response to $^1O_2$ in the facultative photosynthetic bacterium *Rhodobacter sphaeroides*.

Sources of $^1O_2$.

During solar energy capture by *R. sphaeroides* and other photosynthetic cells, chlorophyll pigments within light harvesting complexes are excited to a triplet state (4, 10). At a significant rate, these triplet state pigments transfer energy to O_2 to generate $^1O_2$ (4, 10). Other sources of $^1O_2$ in photosynthetic and non-photosynthetic cells include NADH oxidase, myloperoxidase or chloroperoxidase (5, 10). Although formation of $^1O_2$ alters prokaryotic and eukaryotic gene expression, relatively little is known about the cellular, transcriptional and stress responses to this compound (10).

R. sphaeroides σ^E activates a stress response to $^1O_2$.

Activity of the alternative sigma factor, σ^E, increases when $^1O_2$ is generated *in vivo* (2). *R. sphaeroides* σ^E activity is not increased by superoxide, hydrogen peroxide or hydroxyl radicals (2), suggesting it

controls a specific transcriptional response to $^1O_2$. *R. sphaeroides* σ^E is a member of the extracytoplasmic function, ECF, sigma factor family. *R. sphaeroides* σ^E is like other ECF family members (6, 7) since it forms a complex with ChrR, an anti-sigma factor encoded by the second gene in the *rpoEchrR* operon (12).

ChrR inhbits *R. sphaeroides* σ^E activity.

Some anti-sigma factors directly sense an inducing signal, causing dissociation of the sigma/anti-sigma complex and increased target gene expression (7). Thus, the ability of $^1O_2$ to increase *R. sphaeroides* σ^E activity *in vivo* led to the proposal that this reactive oxygen species somehow causes dissociation of the σ^E-ChrR complex (2), releasing σ^E so it can bind core RNA polymerase and activate gene expression (1). ChrR is a metalloprotein that requires zinc to bind σ^E and inhibit its activity (11). It is unknown if zinc release from ChrR in the presence of $^1O_2$ regulates its function, as is the case in the zinc-containing chaperone Hsp33 and the zinc-containing *Streptomyces coelicolor* anti-sigma factor RsrA, which each respond to other reactive oxygen species (3, 13, 15).

R. sphaeroides σ^E is required for viability in the presence of $^1O_2$.

In phototrophs like *R. sphaeroides,* carotenoids are a line of defense against $^1O_2$ (4, 10). However, $^1O_2$ destroys function of the photosynthetic apparatus, so quenching by carotenoids must be insufficient to totally protect cells (10). $^1O_2$ is bacteriocidal to *R. sphaeroides* σ^E mutants (2), suggesting that one or more σ^E-dependent gene products are required for survival under these conditions.

Conservation of the $^1O_2$ stress response.

The microbial genome database predicts that many other bacteria, including animal and plant pathogens contain homologs of *R. sphaeroides* σ^E and ChrR, presumably to respond to $^1O_2$ generated as a host defense against these bacteria. In addition, structural genes for many other ECF sigma factors are predicted to be co-transcribed with a gene encoding an zinc-metalloprotein. Thus, the analysis of the *R. sphaeroides* σ^E-ChrR pathway will provide information on the function of related proteins in other bacteria.

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