

Photooxidation, Antioxidants, and Photosynthesis in cyanobacteria: Fundamentals to Applications

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Production of Biofuels is a new form of agriculture that calls for new technology and new feedstock organisms. Growing algae for biodiesel would require less land and water for biofuel production than crop plants. Land for growing algae can be of poor quality.



Cyanotech Corporation facility , Kona, HI

Products from algae are potentially diverse and can include biodiesel, starch for ethanol production, animal feed, specialty chemicals, and soil amendments.

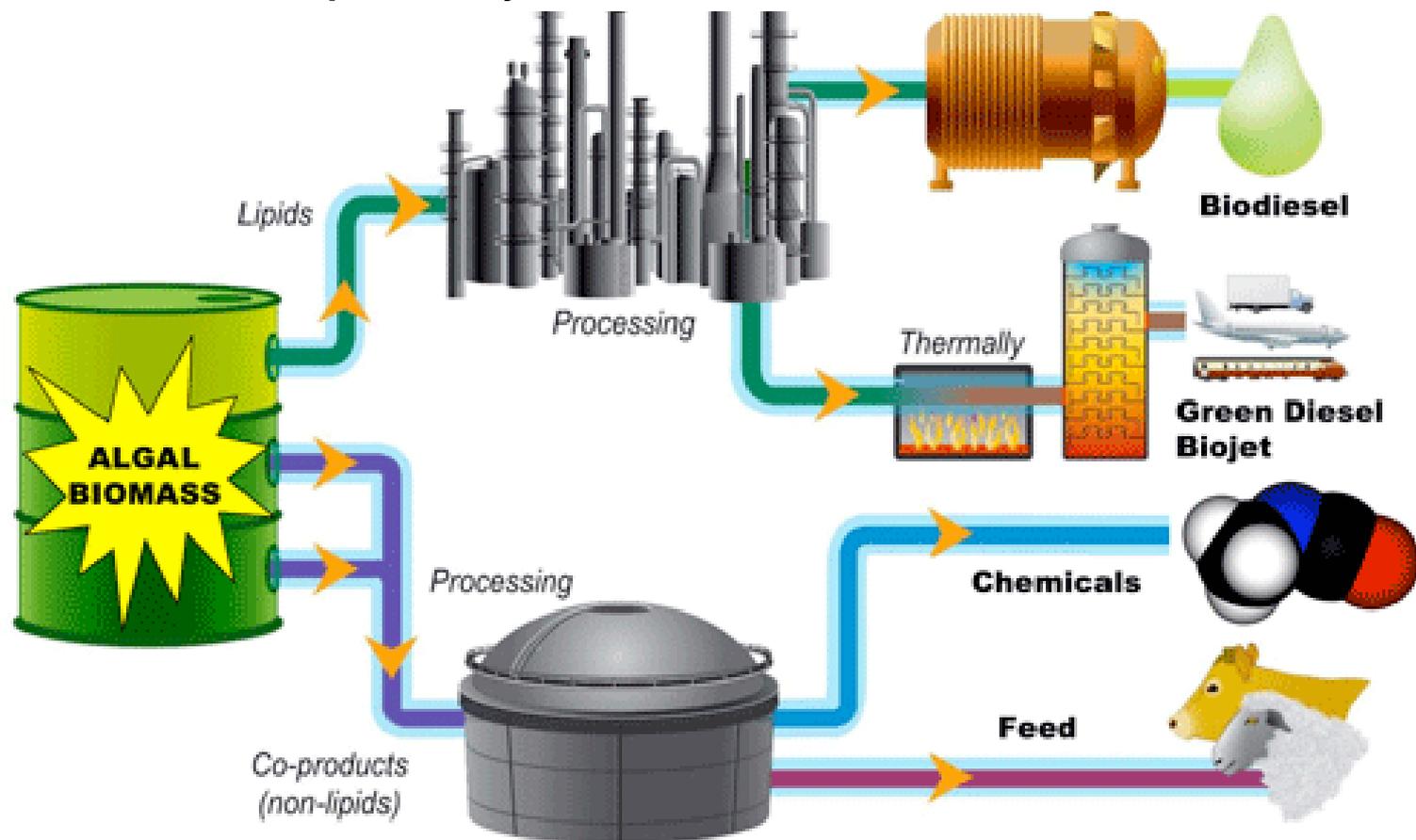
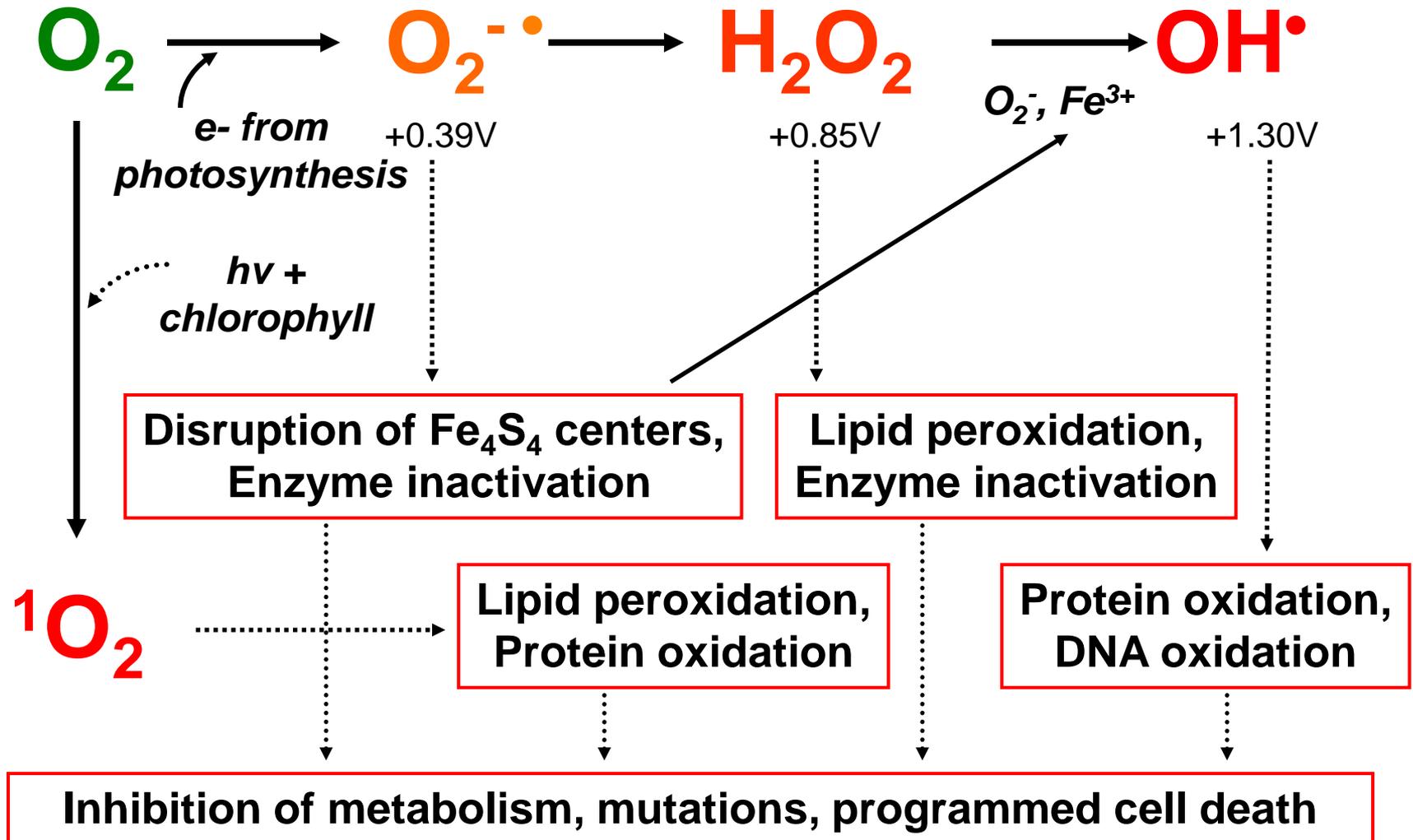


Figure credit: Solix Biofuels at <http://www.solixbiofuels.com/content/products>

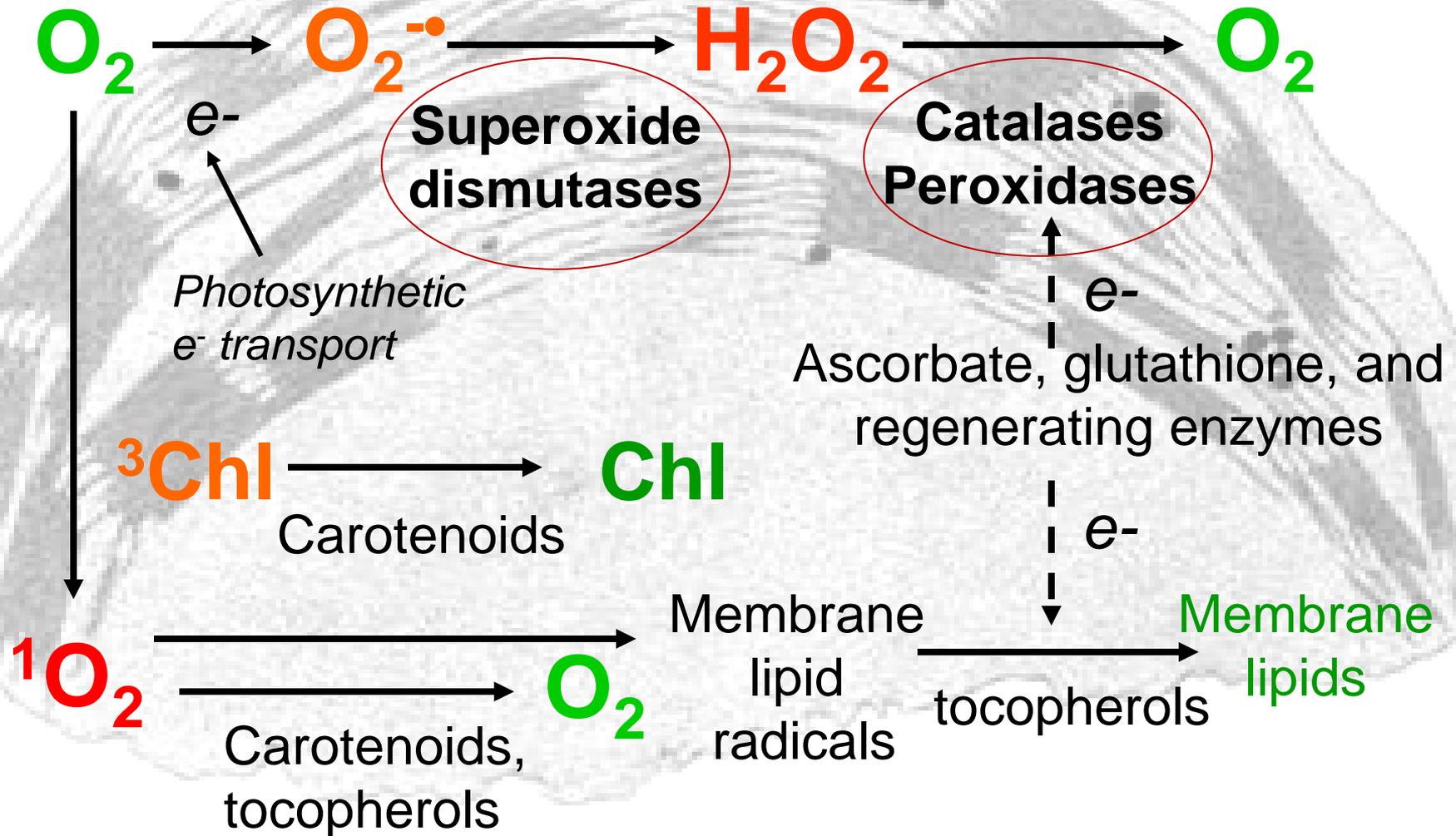


Closed photobioreactor at Arizona State University

Oxidative stress of algal cultures can occur in closed photobioreactors where O_2 can rise to high levels promoting the formation of reactive oxygen species by photosynthesis.



Antioxidant systems detoxify ROS generated by photosynthesis.



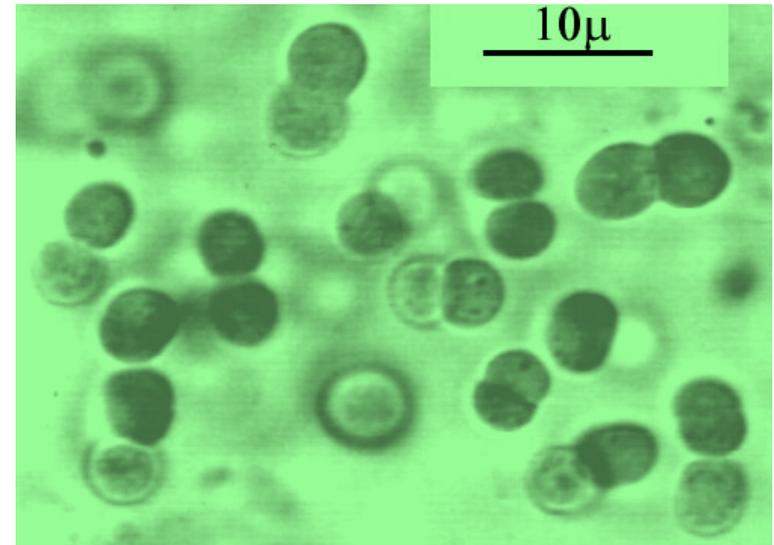
Cyanobacteria are good models for study of oxidative stress in photosynthetic cells:

Transformation is simple. Genes can be specifically disrupted. Transgenes can be expressed.

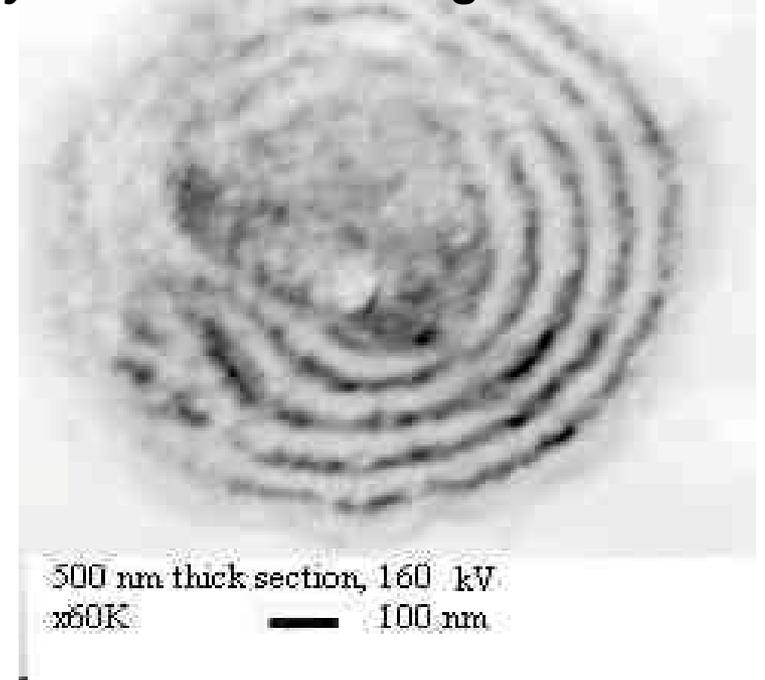
Sequencing of many strains is complete or in progress. Many mutants have been constructed for analysis of gene function.

Lack of a cuticle makes use of chemical inhibitors and cofactors relatively simple.

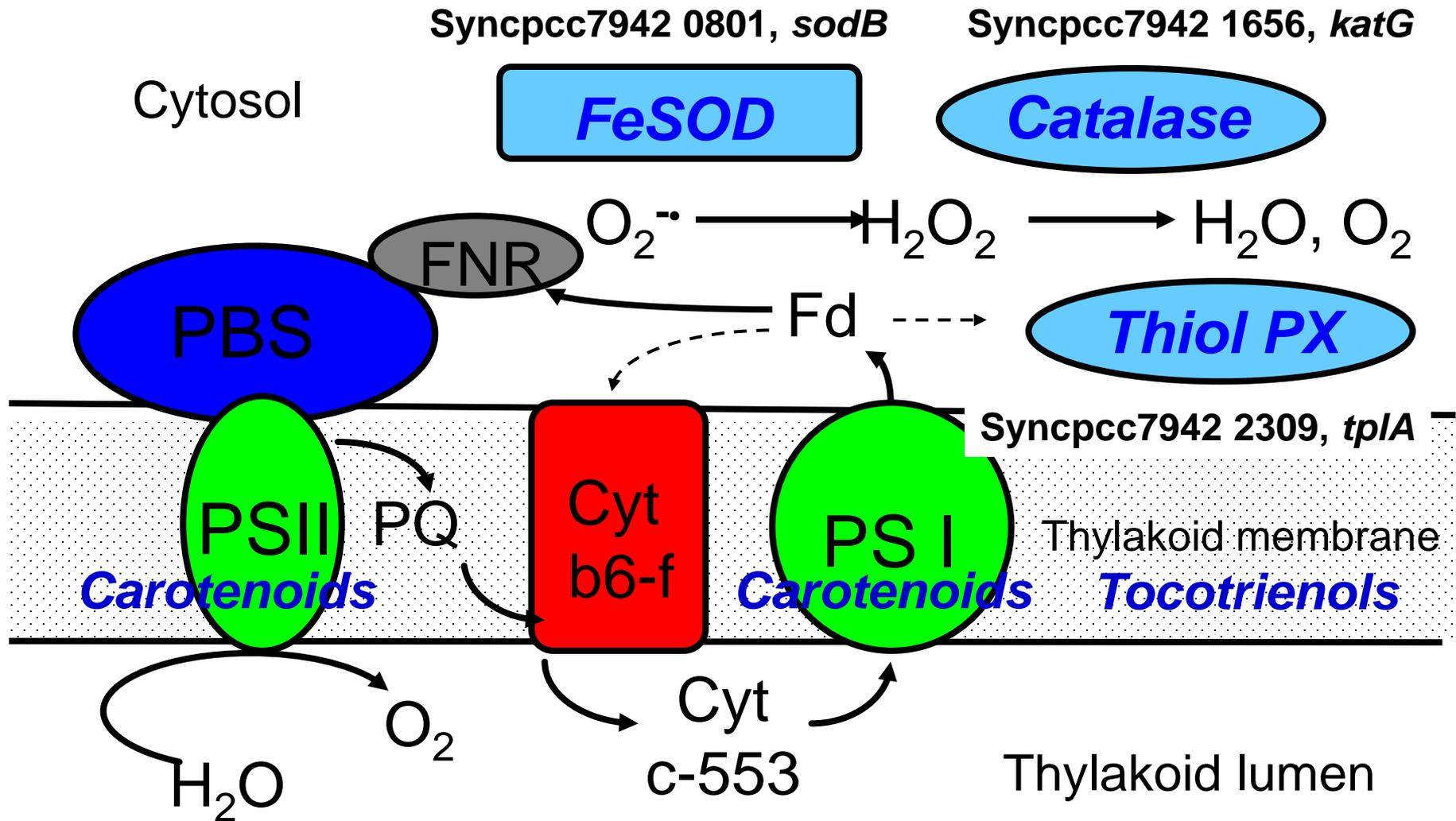
Cyanobacterial genomes and antioxidant systems are simpler than those of eukaryotes, making mutants easier to understand.



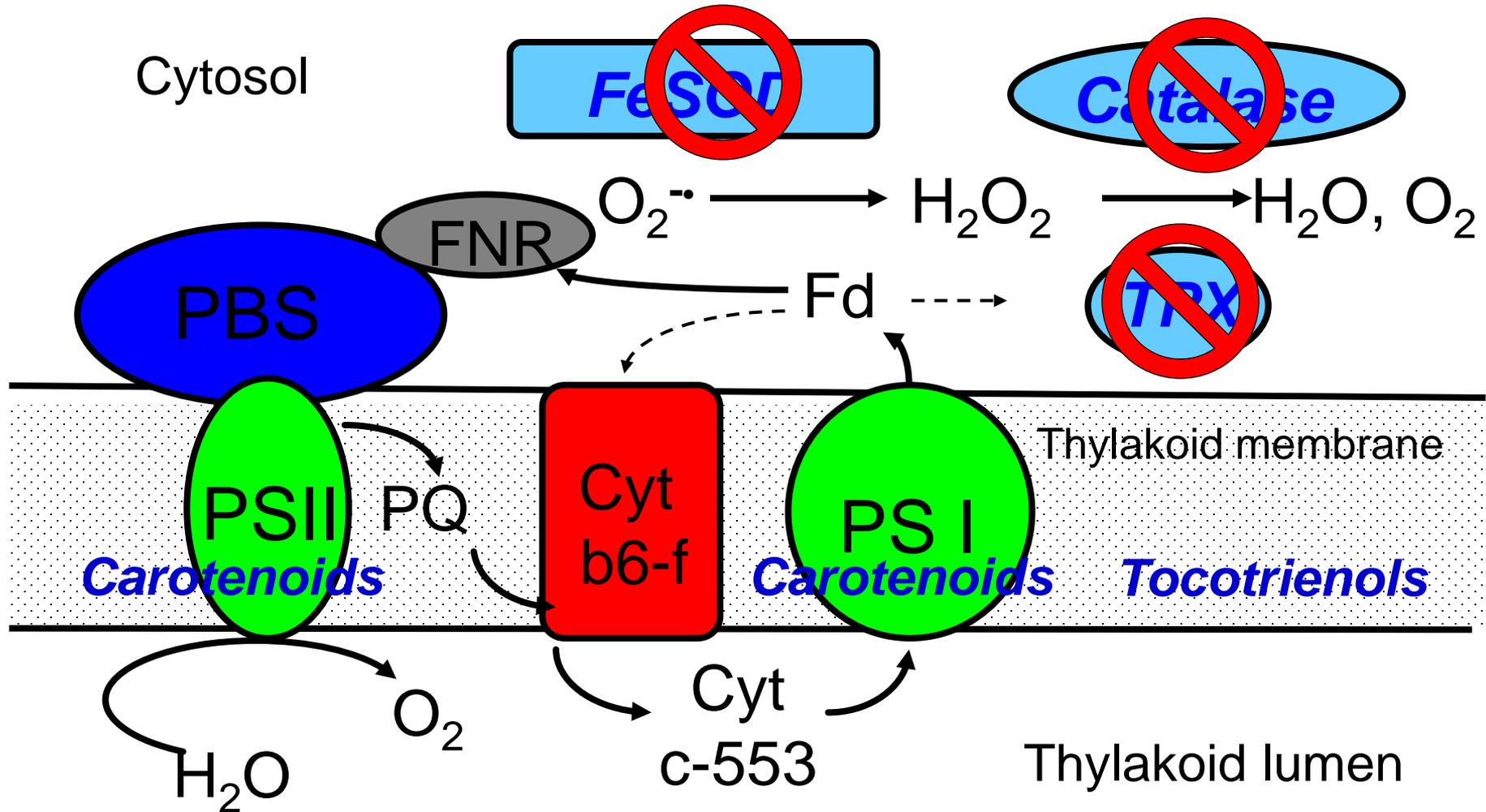
Synechococcus elongatus PCC 7942



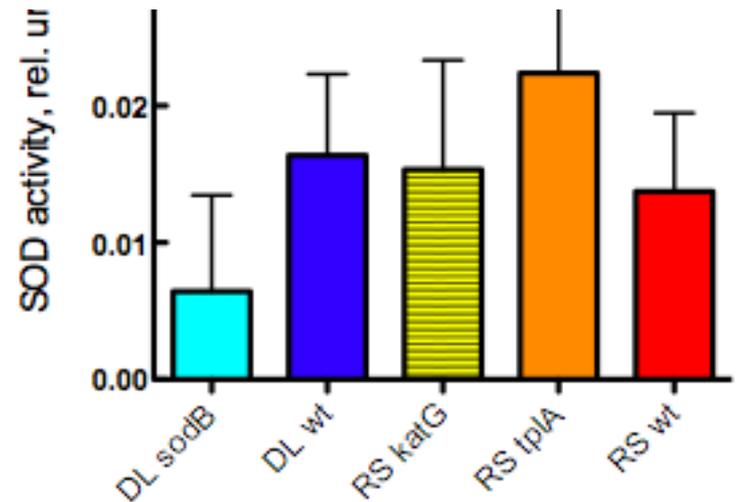
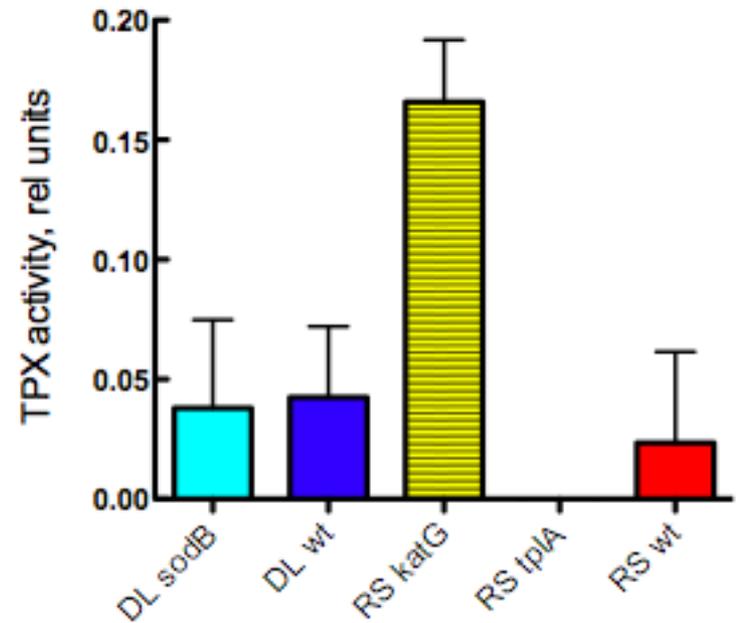
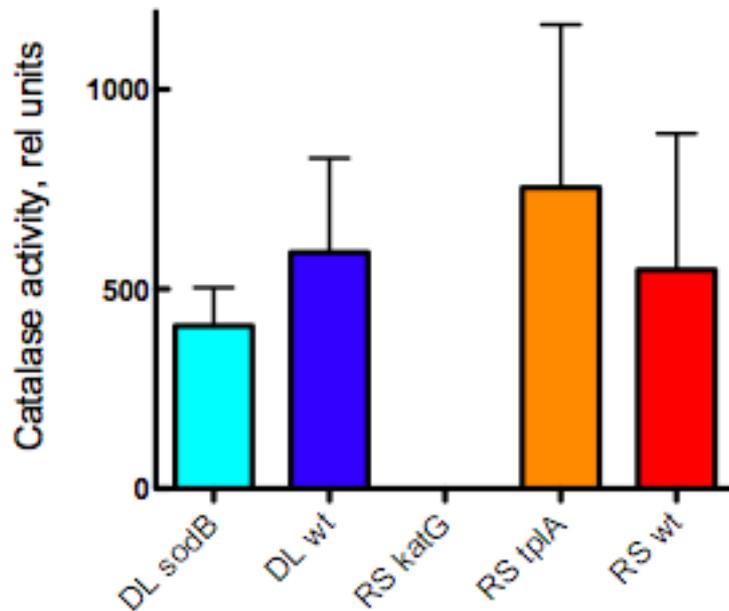
The antioxidant and photosynthetic systems of *Synechococcus elongatus* sp PCC 7942



Single null mutations were made in *sodB*, *katG*, and *tpxA* by site-directed insertional mutagenesis.



Loss of gene function is confirmed with assays of enzyme activity.



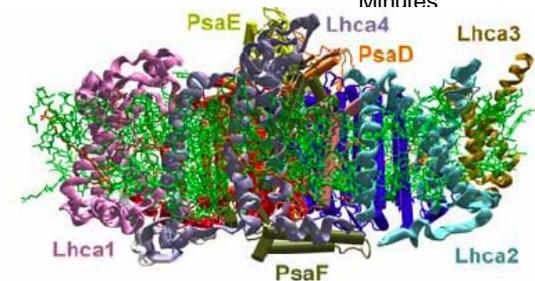
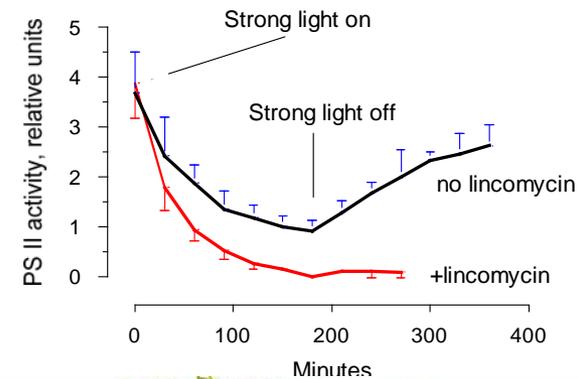
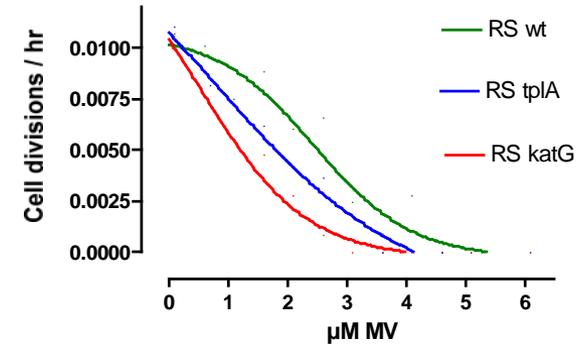
Data are means +/- standard deviations. n= 8 to 10

Phenotype analysis of antioxidant null mutants is proceeding on three tracks.

1. Dose response experiments using treatments that generate reactive oxygen species in defined ways, e.g. paraquat treatment.

2. Comparison of physiological responses to oxidative stress, e.g. photoinhibition of Photosystem II by excessive light.

3. Analysis of important proteins and protein complexes for oxidative damage, e.g. Photosystem I.

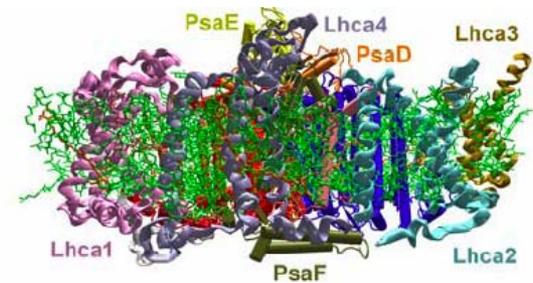
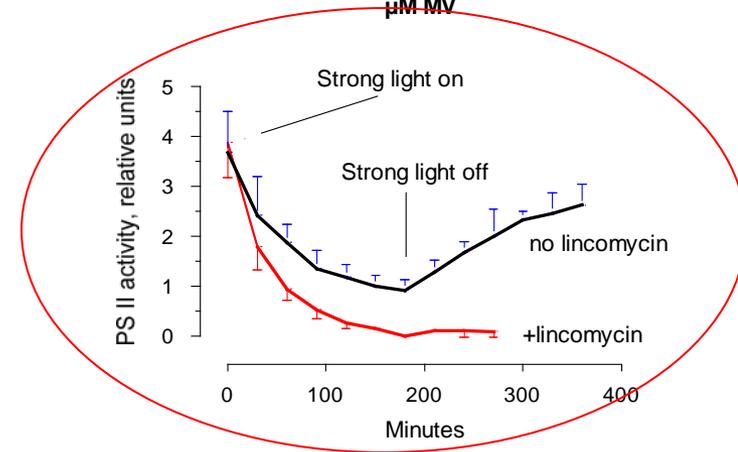
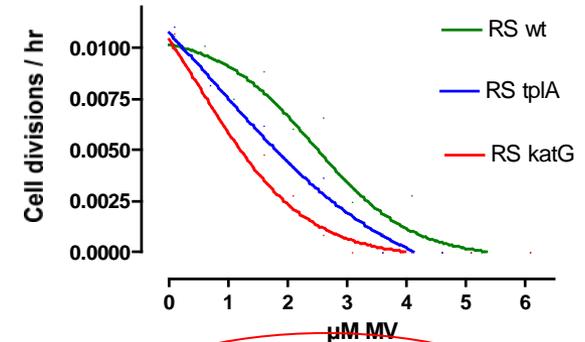


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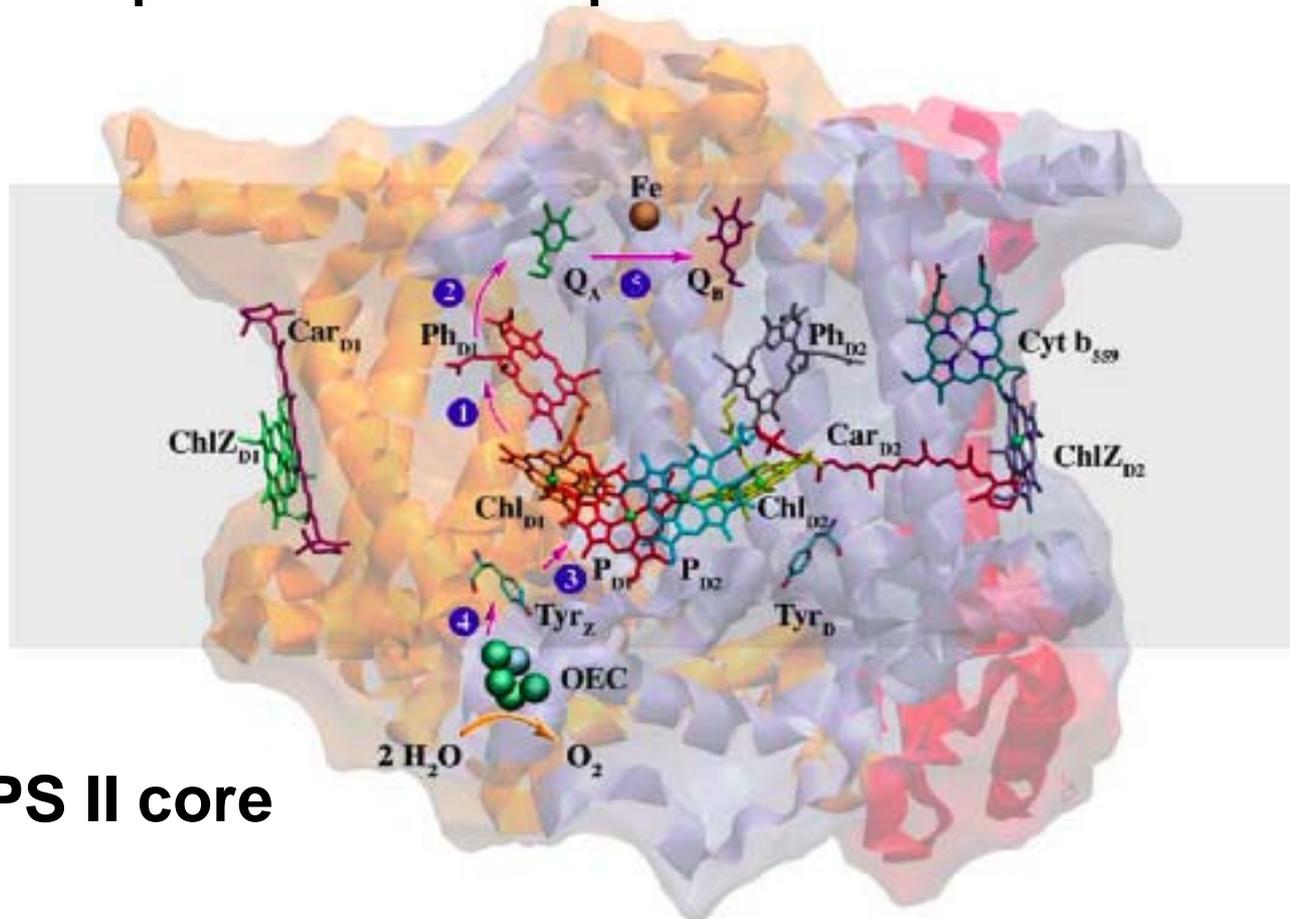
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Photoinhibition of Photosystem II occurs when excitation of PS II exceeds the capacity of downstream e^- transport. Back reactions in the PS II core generate 1O_2 that damages the D1 protein and inactivates PS II. Damaged D1 is removed from the complex and replaced by newly synthesized protein. **Net photoinhibition is the sum of photoinhibition damage and photoinhibition repair.**



PS II core

Photoinhibition is repaired by new protein synthesis.

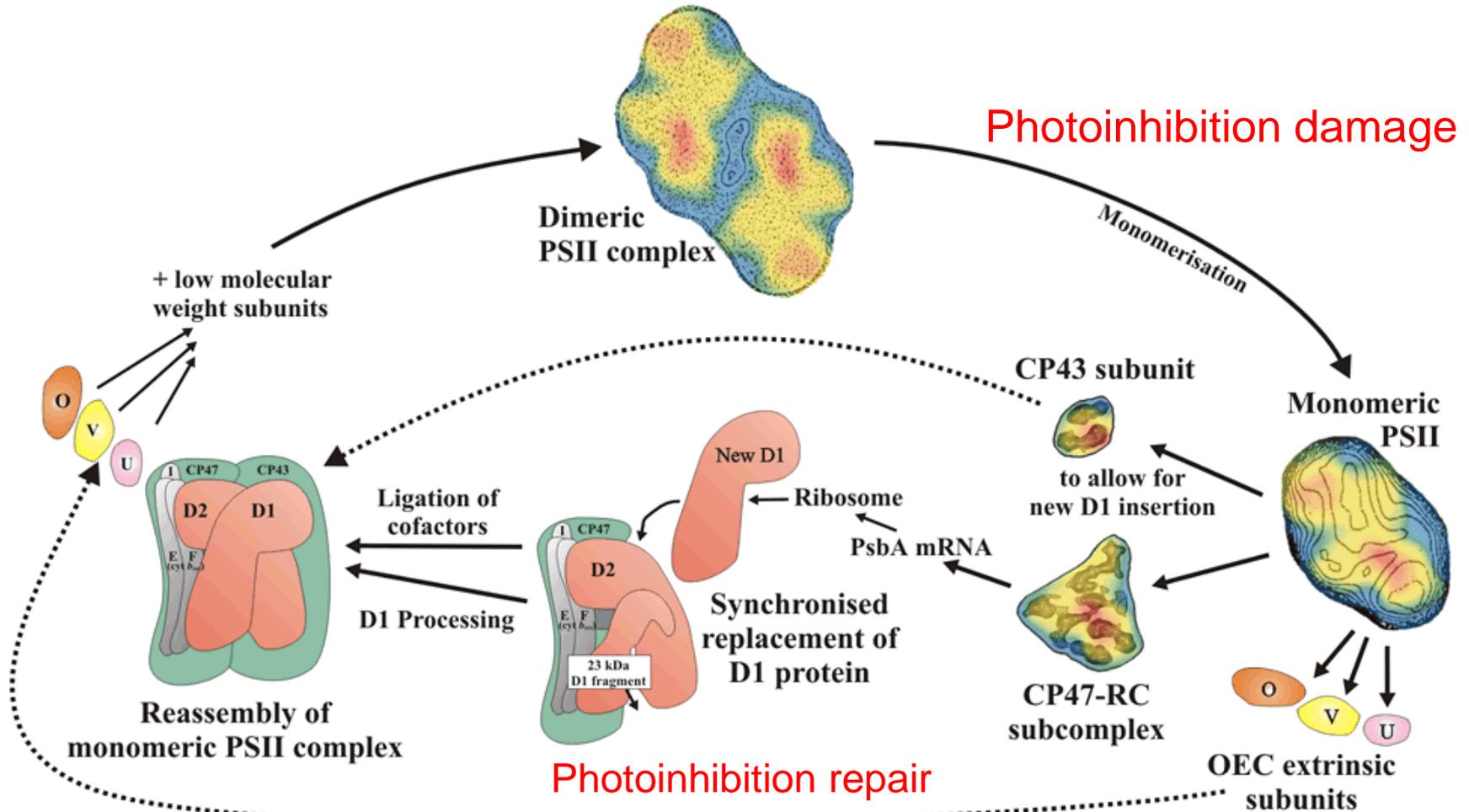
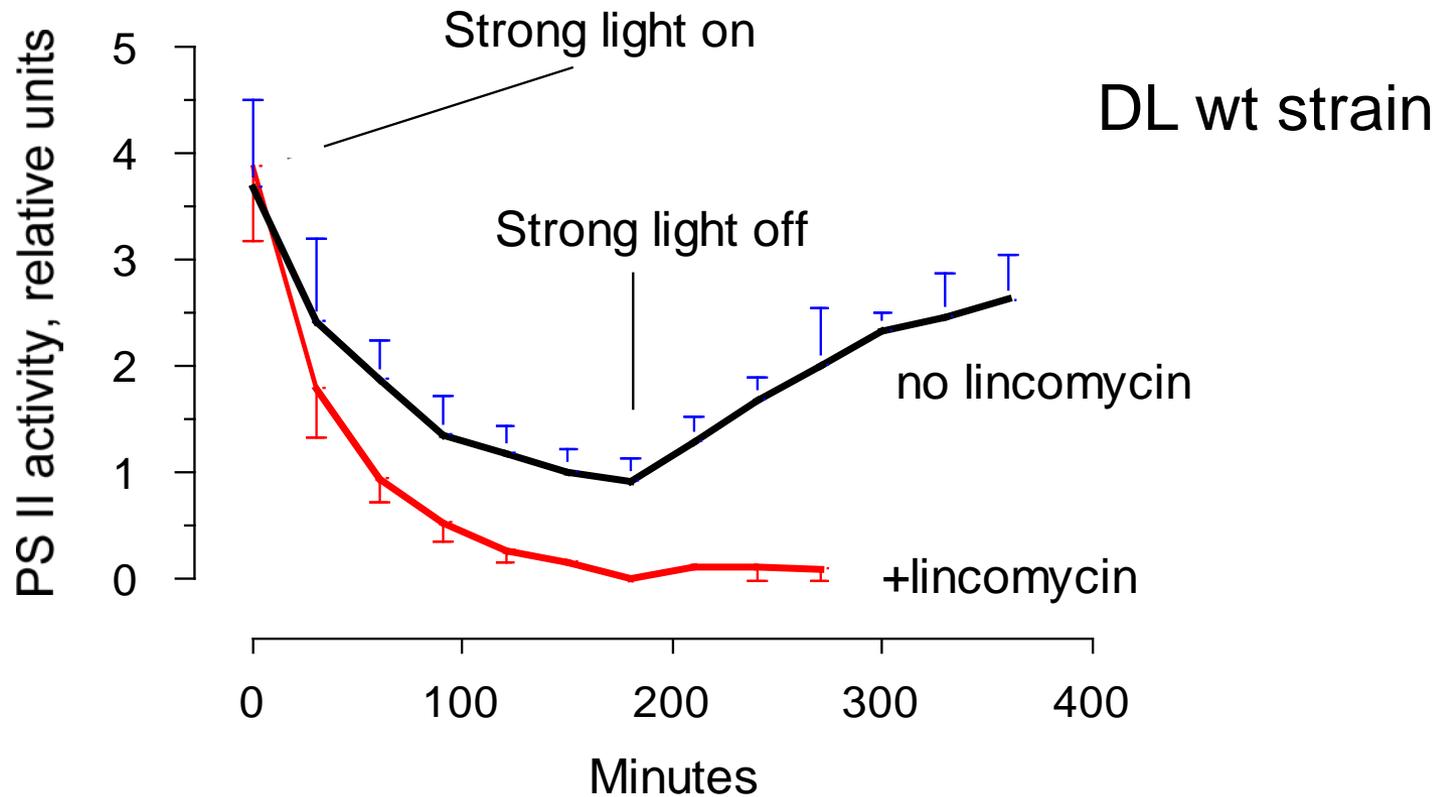


Figure by Peter Nixon, Imperial College, London



Photoinhibition damage and repair assays were performed on mutants and wild types. Lincomycin was used to suppress repair and distinguish it from damage.



Photoinhibition damage and repair parameters. The initial rate of photoinhibition damage was determined from the first 30 minutes of the response to strong light with lincomycin present. The initial rate of photoinhibition repair was determined from the first 30 minutes of the response to strong light without lincomycin minus the response with lincomycin. The equilibrium rate of photosynthesis was the PS II activity remaining after 180 minutes in strong light without lincomycin. The post-stress repair rate was determined from the recovery of PS II activity during the first 30 minutes after the photoinhibition treatment without lincomycin (180 to 210 minutes in Figure 2). Data were analyzed using General Linear Model (GLM) procedure in the Statistical Analysis System [SAS version 9.1.3, SAS Institute 2002-2003] and means were separated using Fisher's least significant difference (LSD) with an $\alpha=0.05$.

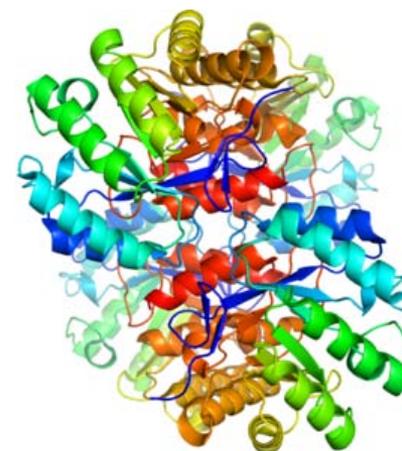
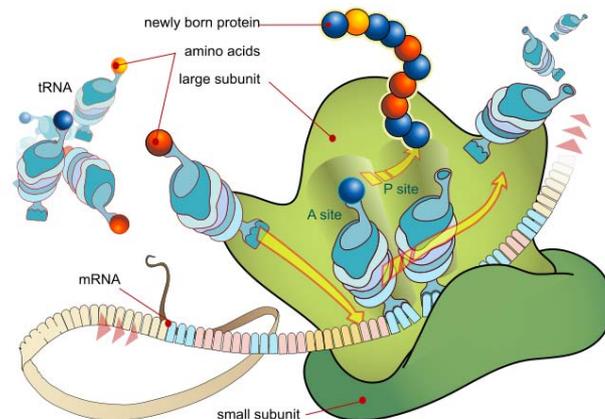
Strain	Initial Rate of damage with lincomycin (%/hr)	Initial Rate of damage, no lincomycin (%/hr)	Initial Rate of Repair (%/hr)	Steady state (%/hr)	Post Stress Repair (%/hr)
RS <i>wt</i>	53.4a	45.8a	7.6	17.8a	7.2a
RS <i>tplA</i>	55.4a	45.0a	10.4	26.2b	12.2b
RS <i>katG</i>	52.4a	45.3a	7.1	20.8a	10.5ab
DL <i>wt</i>	54.2a	34.8b	19.4	25.5b	10.2ab
DL <i>sodB</i>	51.6a	44.8a	6.8	12.0c	2.9c



Conclusions: Photoinhibition damage is unaffected by loss of genes for antioxidant enzymes. Photoinhibition repair is strongly promoted by *sodB* function but not function of *katG* or *tplA*

Current hypothesis: Oxidative stress inhibits photoinhibition repair by damage to elongation factor G of the ribosomal complex. This slows D1 translation (Takahashi & Murata, 2008, *Trends in Plant Sci.* 13:178).

Alternate hypothesis: Stress inhibits photoinhibition repair by inactivation of enzymes required for amino acid synthesis, e.g. dehydratases in the shikimate path. SOD null mutants of yeast and *E. coli* are often amino acid auxotrophs.



Metabolite profiling will show if patterns of soluble amino acids differ between mutants and wild types after photoinhibition, as the alternate hypothesis predicts.

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Samp: GCMS test
Mode: EI +Q1MS LMR LOST UP LR
Oper: Basile
Peak: 1000.0 mmu

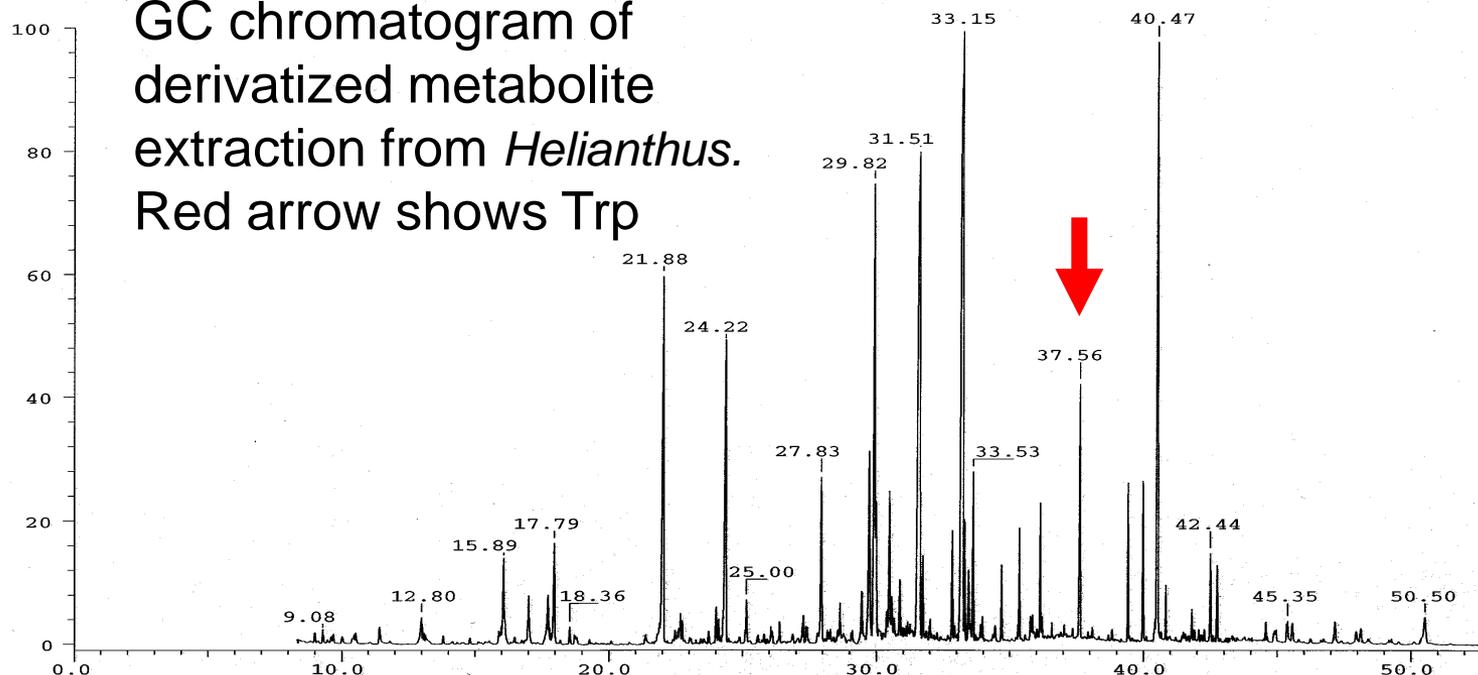
Study: Amino Acid Analysis
RIC: 23343033

Elapse: 1 @ 8.08
Times: 8.1 > 53.0
Inlet: GC Vial: 2
Client: Univ. of Wyoming
Masses: 50 > 550

RIC

2.3E+07

GC chromatogram of derivatized metabolite extraction from *Helianthus*.
Red arrow shows Trp



Over expression of genes for antioxidants and amino acid biosynthesis could improve yields of algae in large scale culture, especially if cultures are fertilized with CO₂ emissions from coal-fired power plants like cement plants.



Mountain Cement Corporation, Laramie, WY.

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