Redesigning Living Organisms to Survive on Mars

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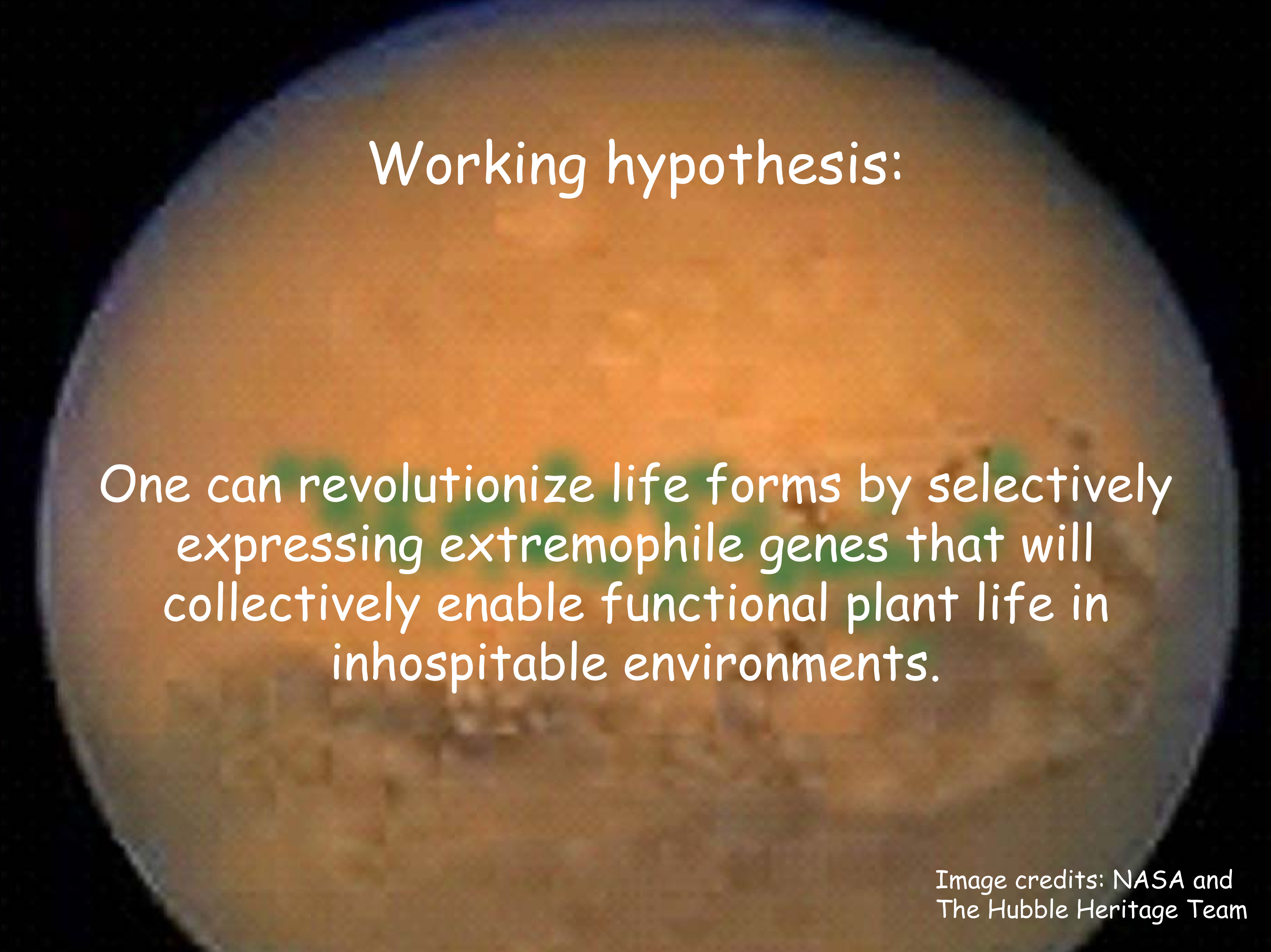
Proof of concept:

A. To produce a functional extremophilic protein in plants

B. To engage undergraduates in developing a preliminary design for virtual plants that would survive on Mars

Image credits: NASA and The Hubble Heritage Team



Working hypothesis:

One can revolutionize life forms by selectively expressing extremophile genes that will collectively enable functional plant life in inhospitable environments.

Image credits: NASA and The Hubble Heritage Team

Why Plants?

Plants Provide:

Bioregenerative life support for long term space exploration

Medicines, building materials, fuel and a positive psychological environment

Test system for long term survival that will not generate public outcry if the plants die

A basis for terra-forming

Challenges posed by the Martian Environment for Plant Growth

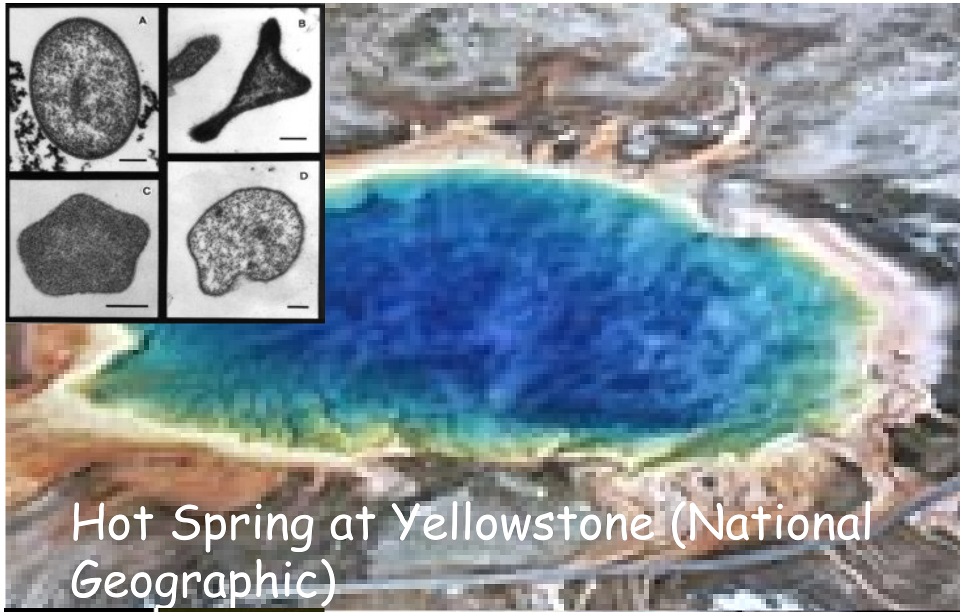
Lack of Water
Lack of O_2
Low pressure (about 6 mbars)
Extremely Cold Temperatures (-125 to -5°C)
UV and cosmic Radiation
Limited mineral nutrients

Image credits: NASA and The Hubble Heritage Team

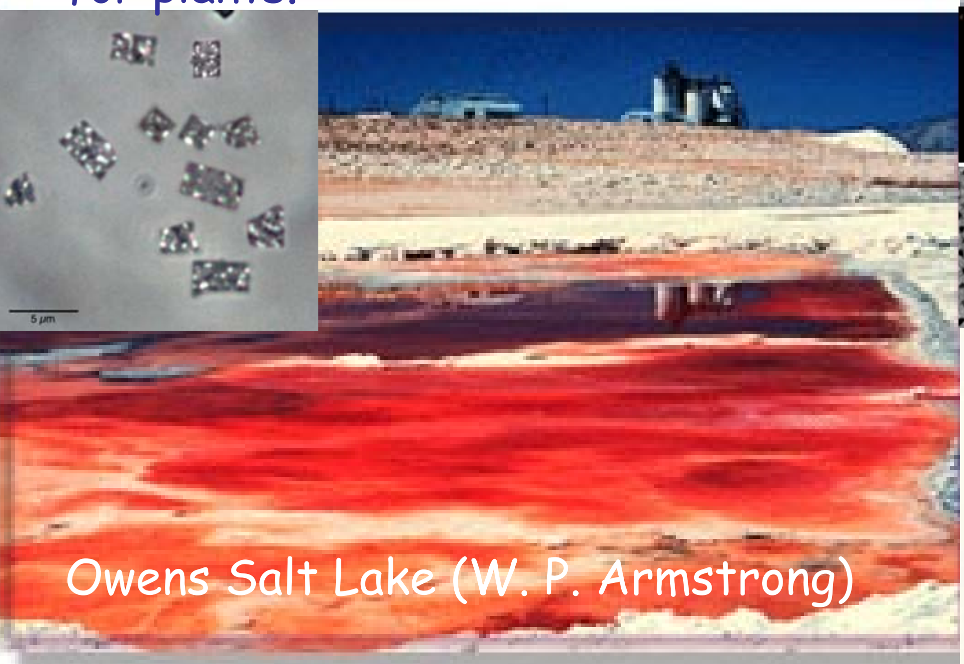
Why Extremophiles?

The conditions on Mars are beyond what plants as we know them can survive and provide a bioregenerative life support system for human exploration.

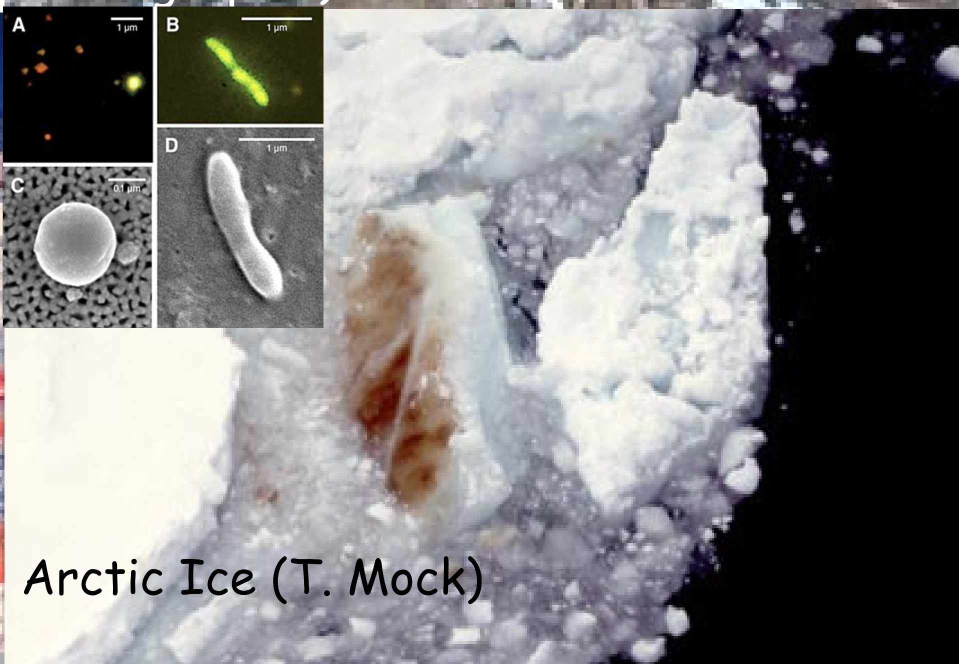
By combining strategies used by microorganisms which can survive and thrive in extreme environments we propose to make a major advance in extending the growth environment for plants.



Hot Spring at Yellowstone (National Geographic)



Owens Salt Lake (W. P. Armstrong)



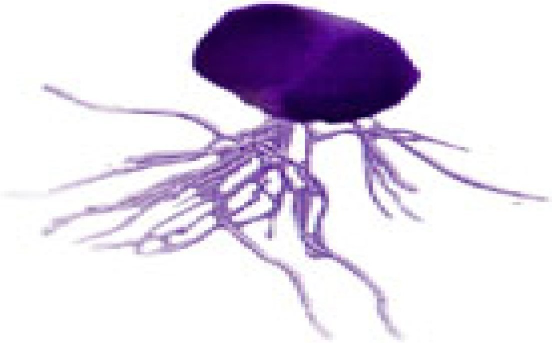
Arctic Ice (T. Mock)

Assumptions and Challenges:

Extremophilic archaeal genes will be transcribed and translated into a functional protein by the plant transcription and translation machinery.

The functional archaeal protein would not harm the plant.

Why *Pyrococcus furiosus* Superoxide Reductase (SOR)?



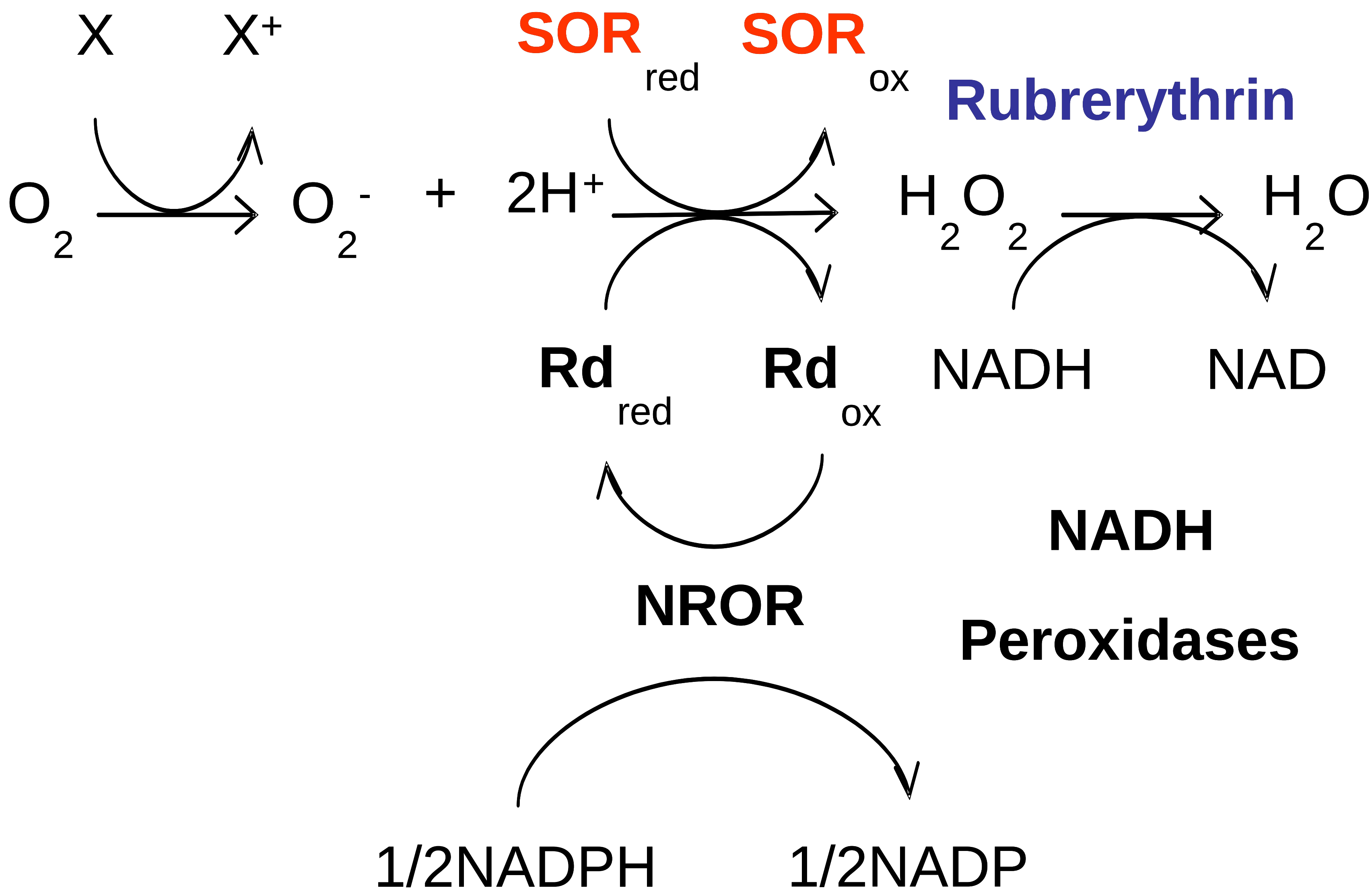
SOR is part of an oxygen detoxification system that could be beneficial to plants under stress conditions.

The pathway can function under both aerobic and anaerobic conditions.

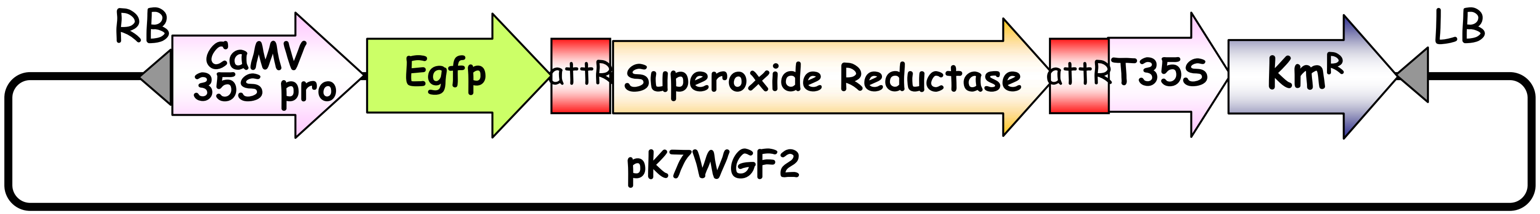
The pathway is not found in plants.

Pyrococcus furiosus SOR is heat stable so that it can be assayed after heat denaturing the plant enzymes.

Superoxide Detoxification System in *Pyrococcus furiosus*



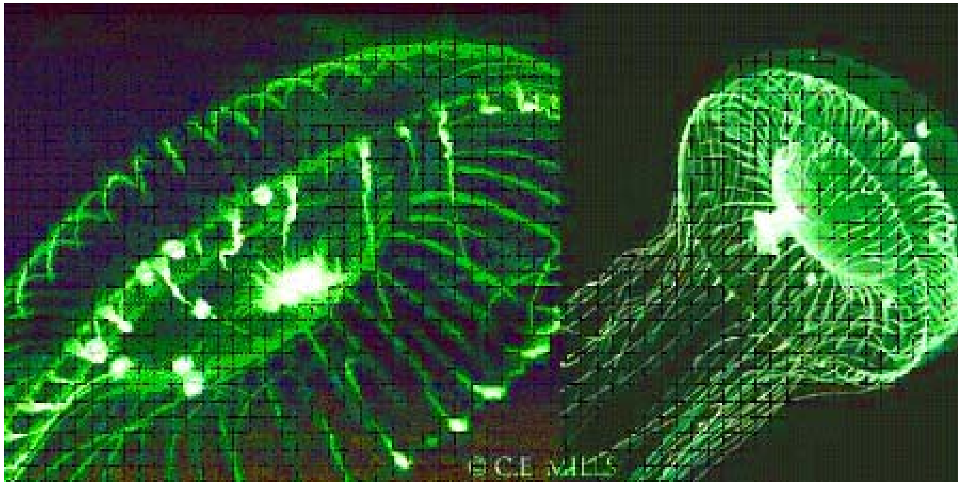
Pyrococcus furiosus SOR DNA was inserted into a Gateway entry vector and then into a binary vector for plant transformation



Provided by the Functional Genomics Division of the Department of Plant Systems Biology

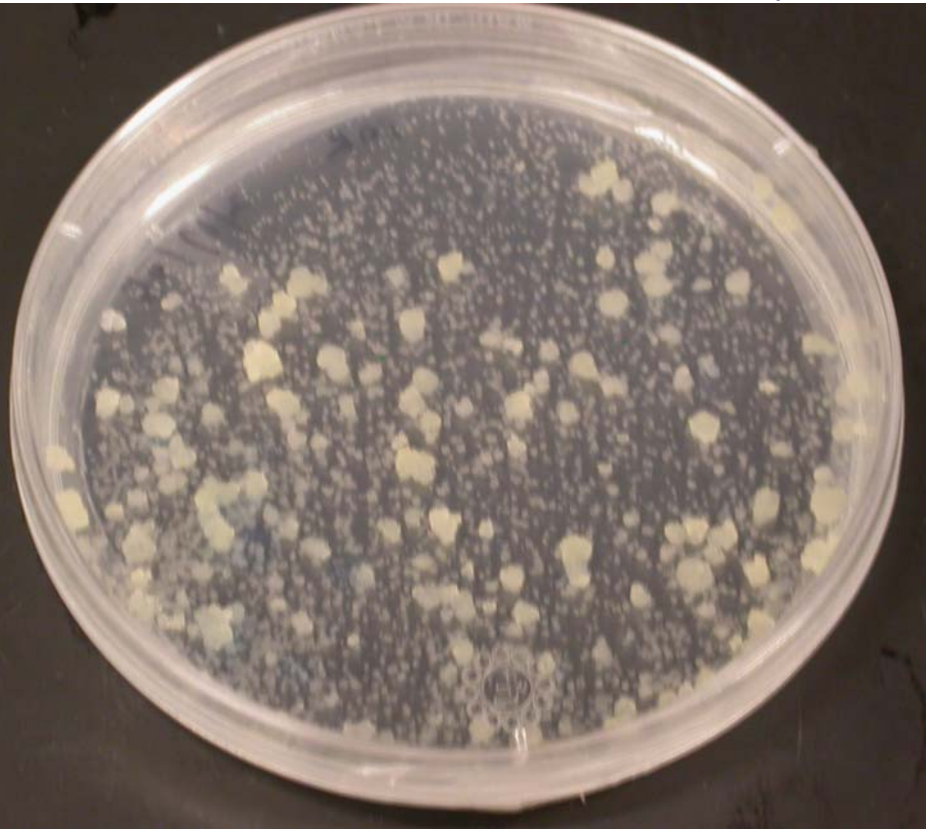
http://www.psb.ugent.be/gateway/index.php

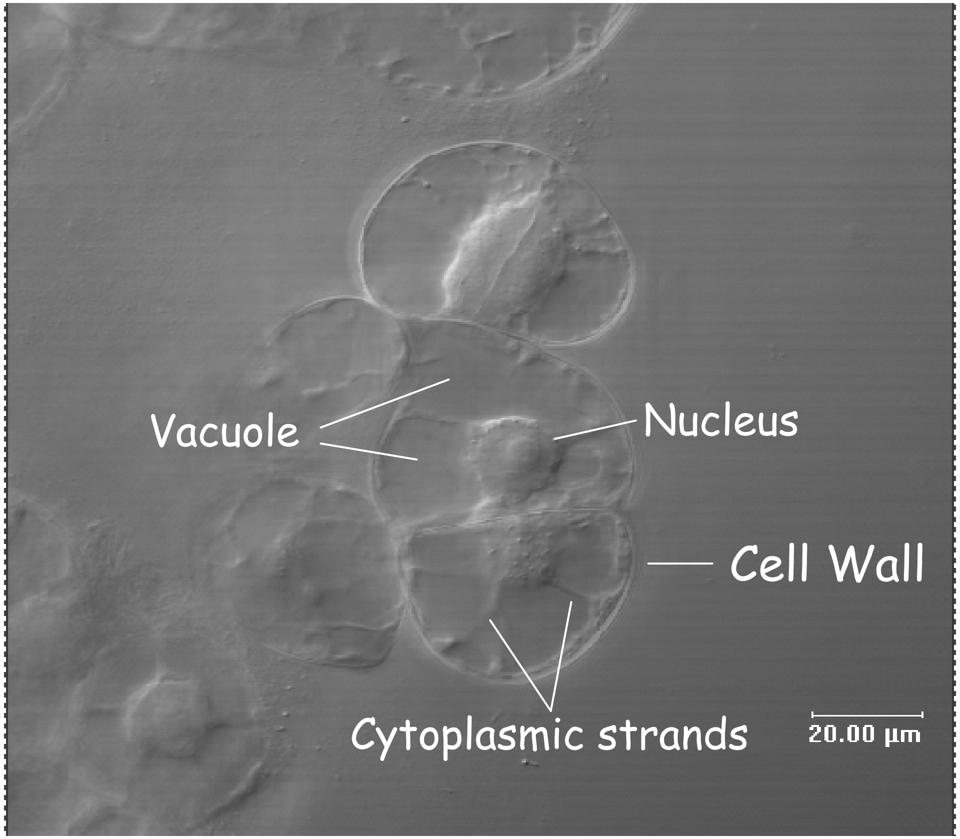
Green fluorescent protein from the Pacific jellyfish, *Aequoria victoria*, when fused to your favorite protein will reveal the subcellular location of the recombinant fusion protein.



Plant Cell Transformation

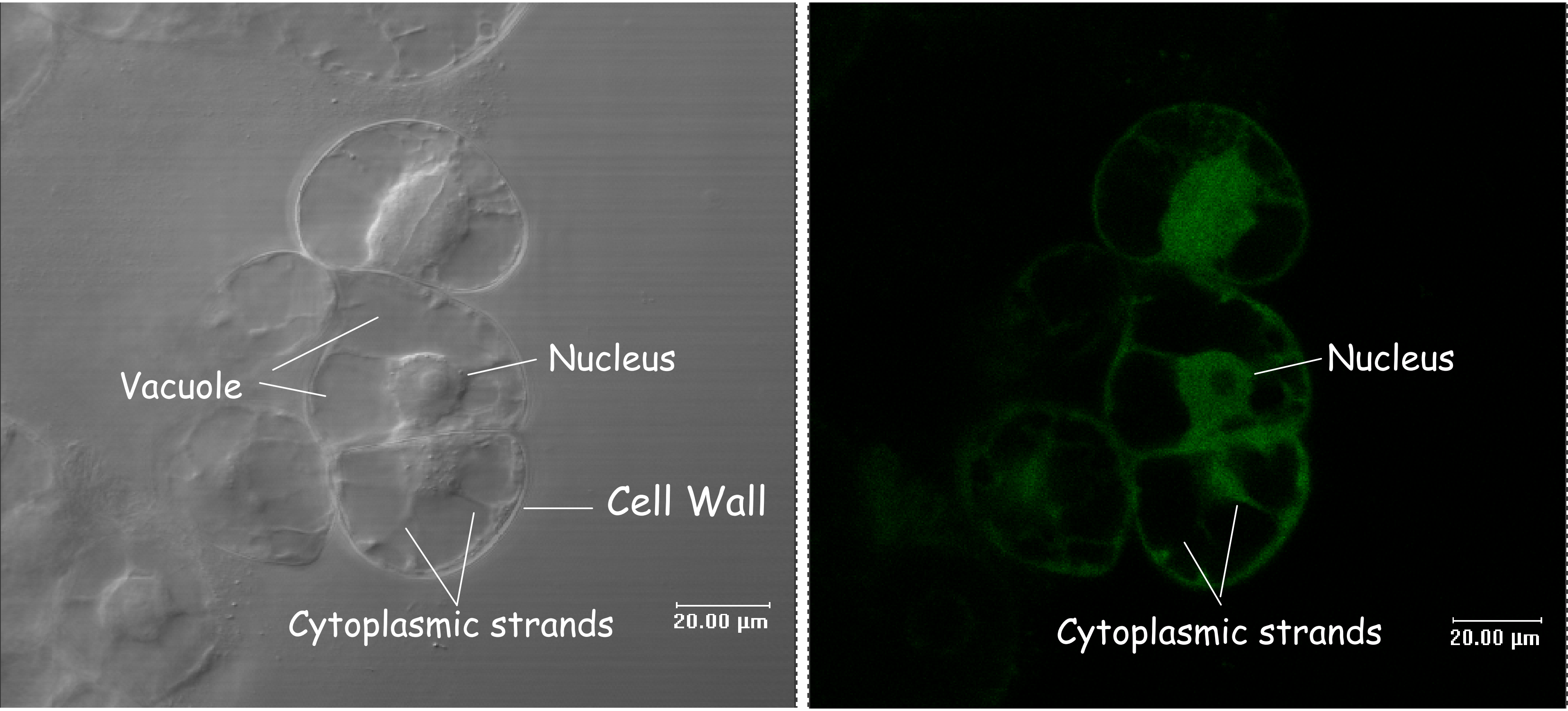
For proof of concept we used a relatively rapid plant transformation system: tobacco cells grown in suspension culture.

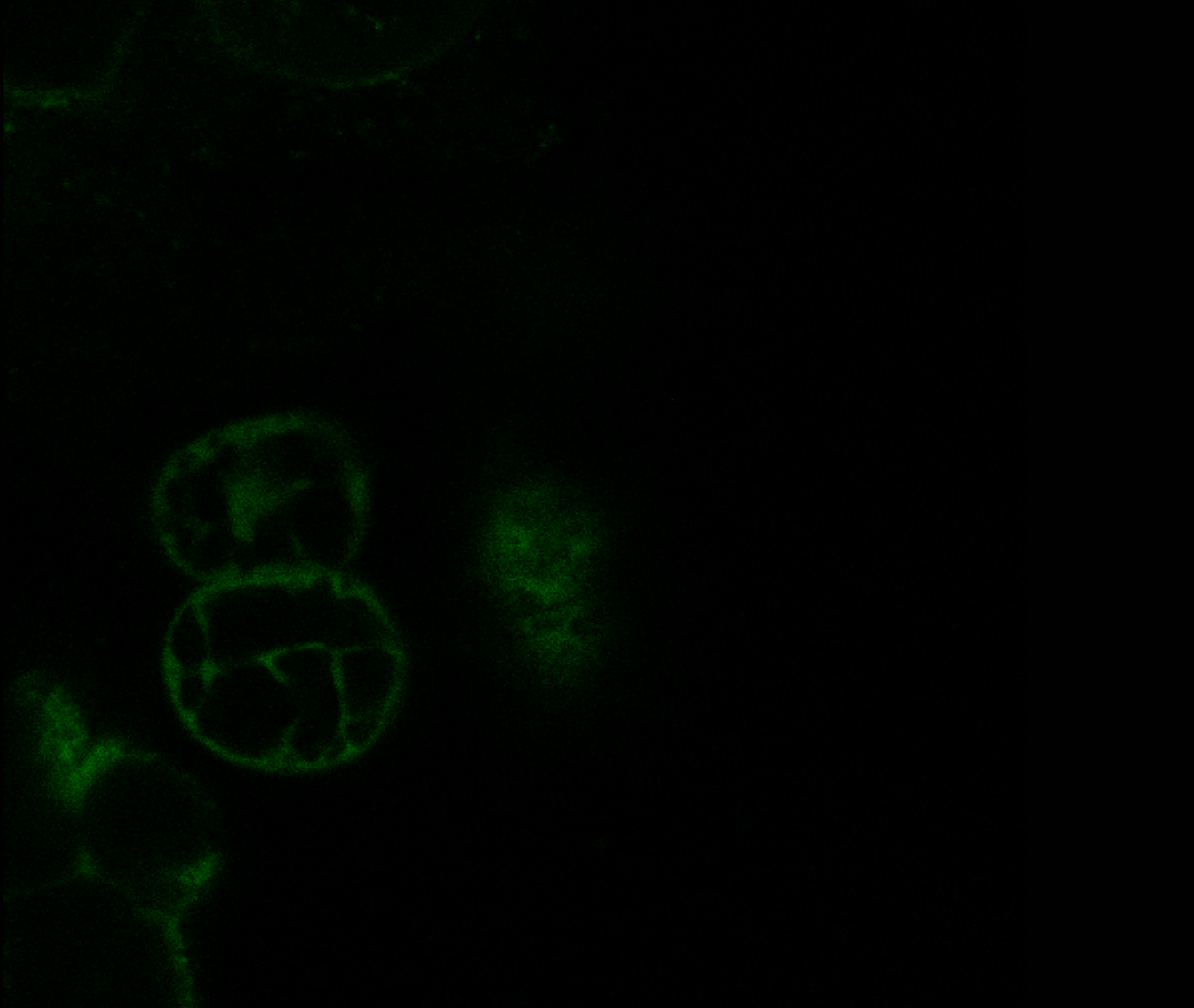




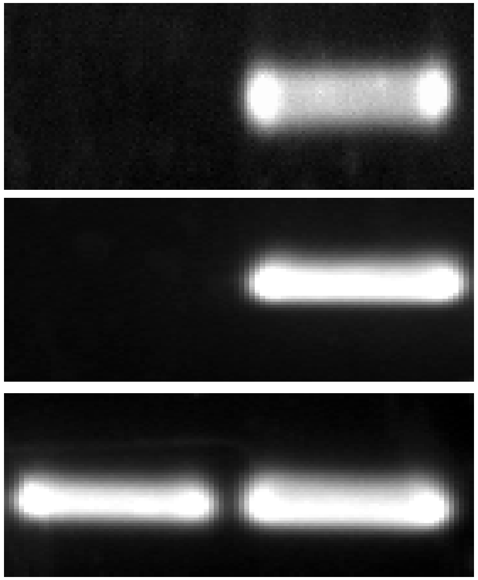
NT1 tobacco cell culture transformed with the Pf-SOR expression plasmid

Pyrocossus furiosus SOR is a soluble protein. GFP-SOR is present throughout the cytosol of the tobacco cells.





mRNA encoding GFP-SOR fusion protein is present in the transformed tobacco cells



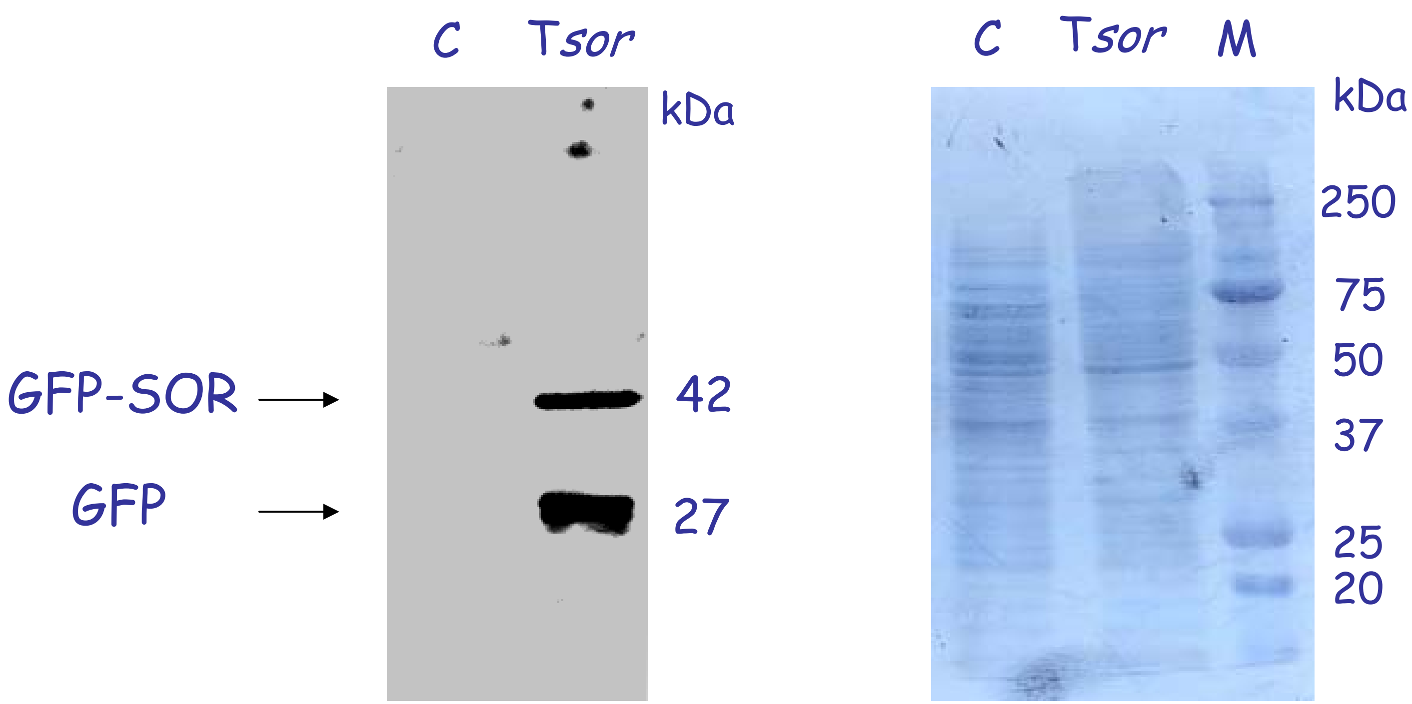
RT-PCR was used to monitor levels of RNA. Specific primers identified *P. furiosus* SOR; GFP and tobacco cell actin (a loading control).

Control (Wild type NT-1 tobacco cells)
T*sor* : Transgenic tobacco cells expressing SOR

The full length GFP-SOR protein can be recovered from the transformed cells.

Immunoblot with
GFP monoclonal antibody

Imido black
Stained blot



C: Control (Wild type NT-1 tobacco cells)

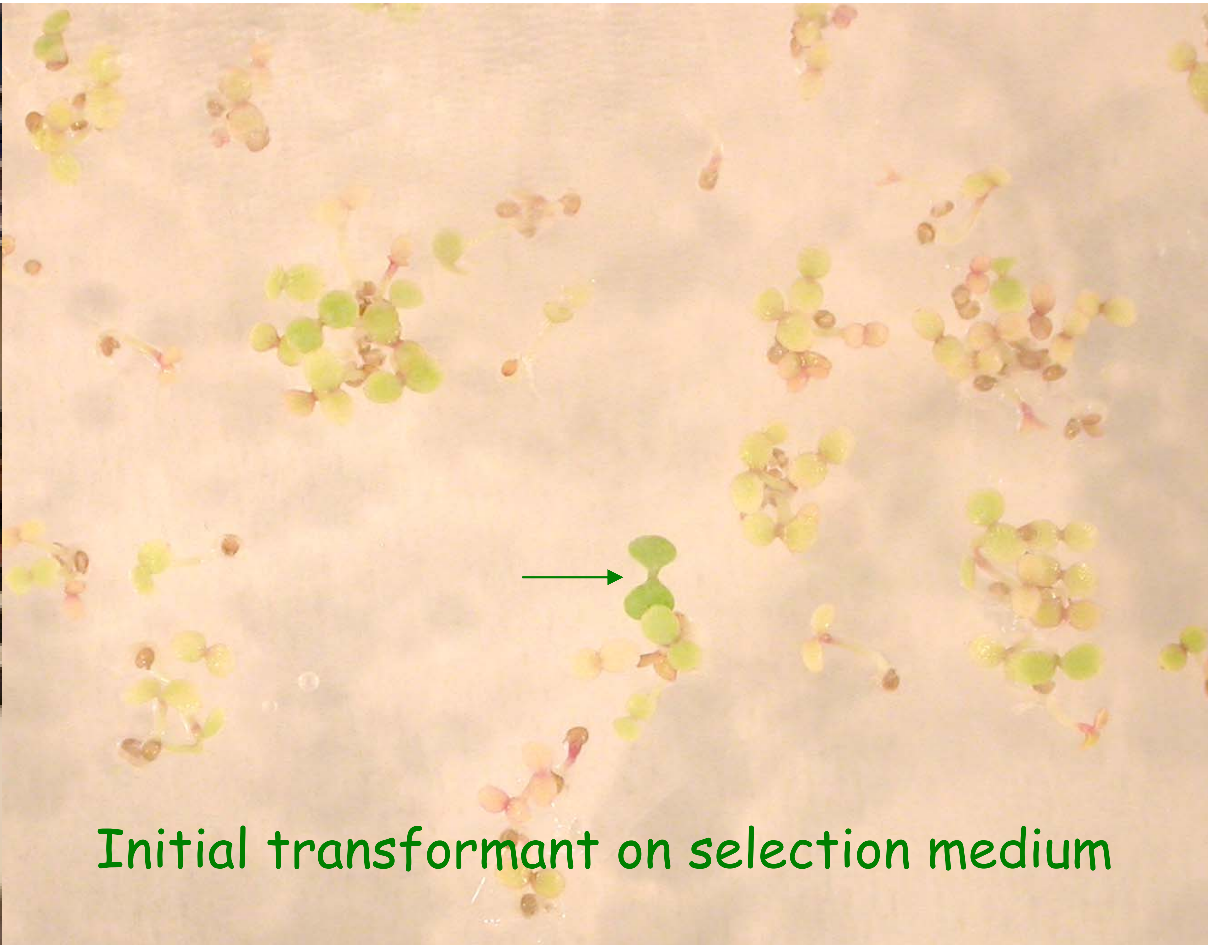
Tsor : Transgenic tobacco cells expressing *P. furiosus* SOR

Recombinant *P. furiosus* SOR is functional when produced in tobacco cells grown in suspension culture

Sample*	Specific Activity (U/mg)
NT1 wild type	Not Detected
NT1-SOR	15.4
NC906-SOR	9.6

* Samples were heat treated at 80°C for 15 min prior to the assay.

The next step is to transform *Arabidopsis thaliana plants* with *P. furiosus* SOR and assess the effects on plant growth.



Challenging undergraduate students to develop a preliminary design for virtual plants that would survive on Mars.

- Developed an Honors class: ALS 398 Redesigning Living Organisms to Survive on Mars– Development of Virtual Plants
- 9 honors students are enrolled in the course
- Students have determined the challenges to life existing on Mars and are developing designs for recombinant plants that would survive on Mars within greenhouses
- Students will present their designs during a mock press conference on April 26, 2005 and as a written research paper

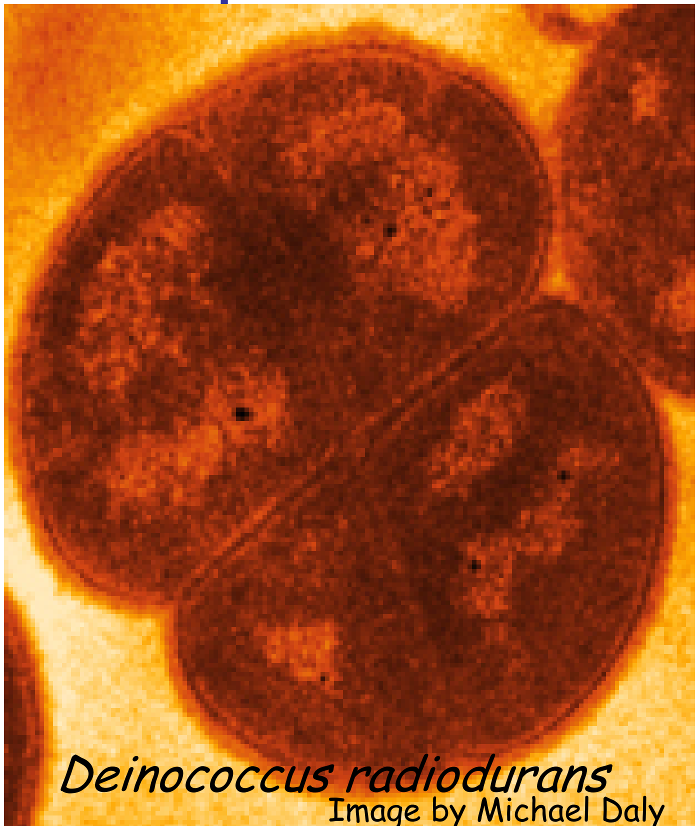
ALS 398 students discussing their plans for redesigned plants

Student Project Groups

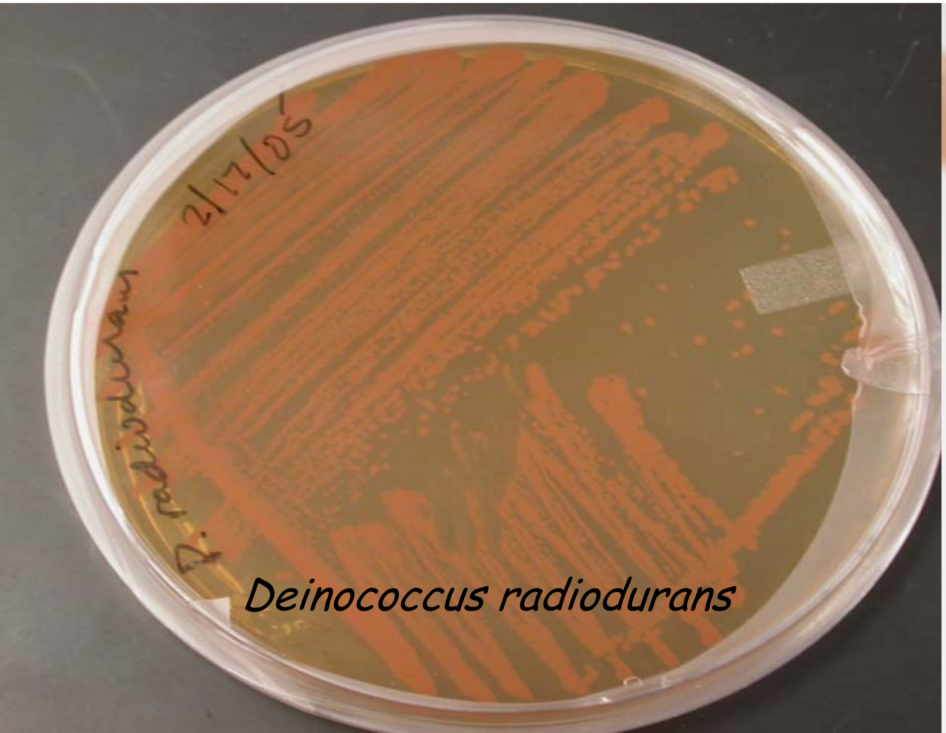
- Students organized themselves into 3 project groups:
  - **Plant Engineering group**
  - **Greenhouse Design/Martian Conditions group**
  - **Project justifications and ramifications group**

The Plant physiology class also became engaged in the project.

- The question they asked was would *Deinococcus radiodurans*, which is resistant to radiation, act as a type of "sunscreen" for plants?



Plant leaves were coated with a solution of *Deinococcus radiodurans* in a wetting agent or wetting agent (control solution) alone. Plants were exposed to 254 nm UV light (10 cm from the plant for 1 h).

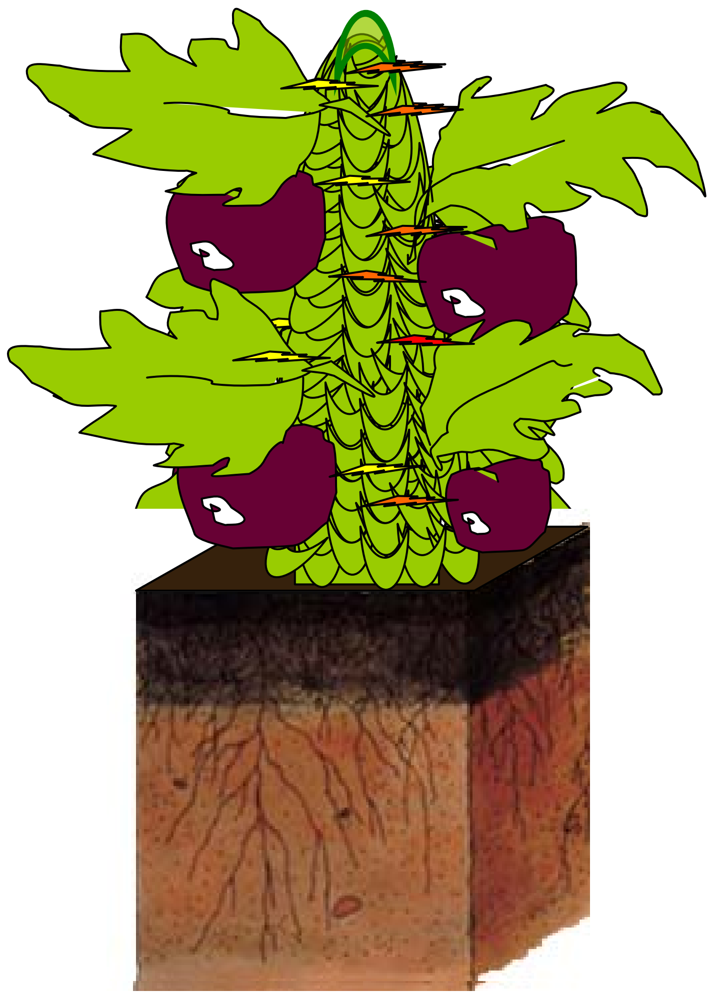


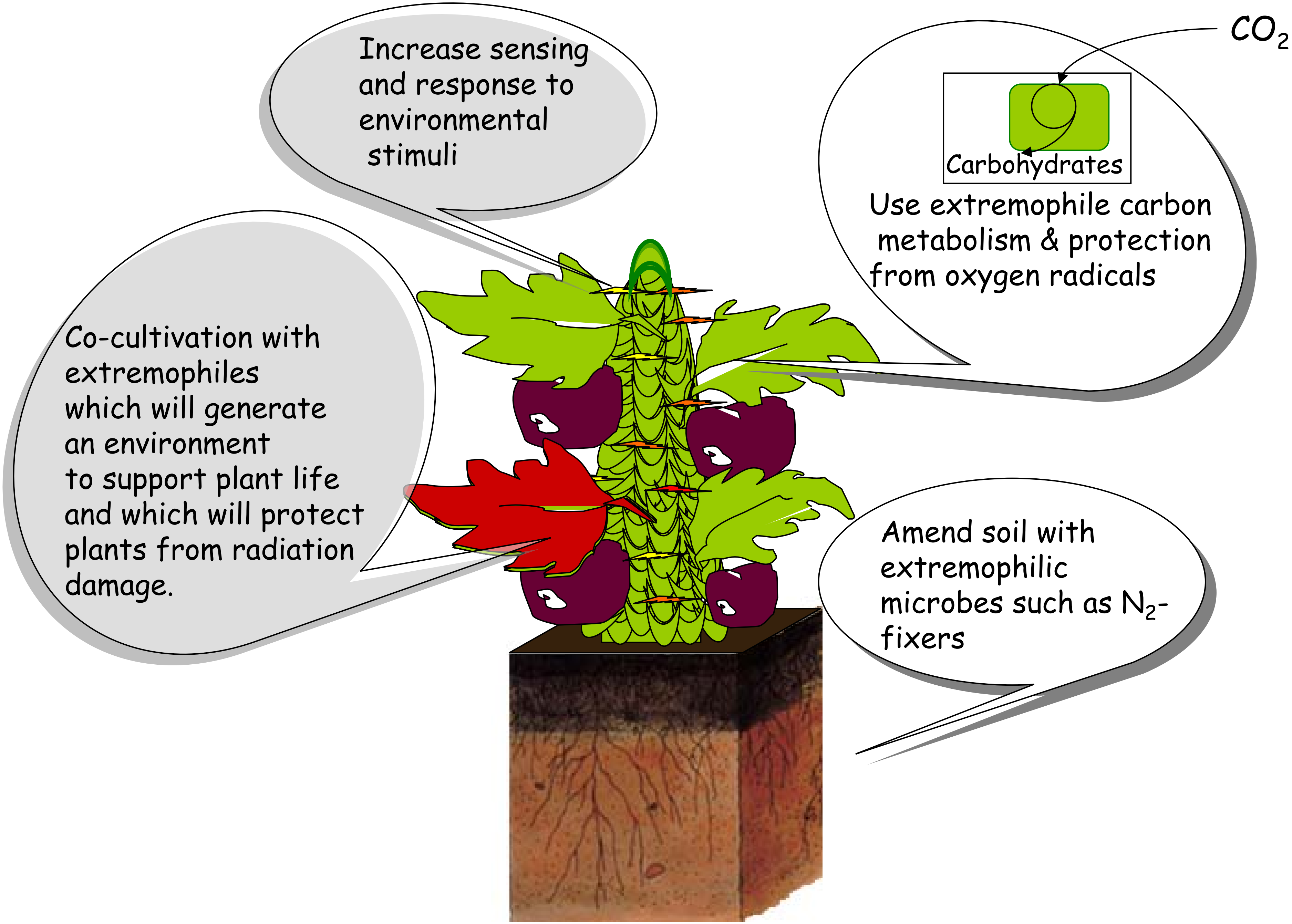
Our goals were to produce a functional archaeal protein in plant cells and to engage undergraduates in designing virtual plants to survive on Mars.

- *Pyrococcus furiosus* superoxide reductase can be produced by plant cells grown in suspension culture.
- The recombinant protein is functional and heat stable.
- Students at NC State are thinking about new ways to engineer plants and plant environments to support plant growth on Mars.

Future Goals

- Transform plants with suite of genes for SOR pathway.
- Transform plants with a suite of genes from the psychrophile *Methanococcus burtonii* to enhance carbon metabolism in the cold and provide increased cold tolerance.
  - Triose phosphate isomerase
  - Fructose-bisphosphate aldolase
  - Phosphoglucomutase
  - Glyceraldehyde-3-phosphate dehydrogenase
  - Phosphoglycerate kinase
  - Enolase
  - Antifreeze protein
  - Compatible solute formation




Increase sensing and response to environmental stimuli
CO_2
Carbohydrates
Use extremophile carbon metabolism & protection from oxygen radicals
Co-cultivation with extremophiles which will generate an environment to support plant life and which will protect plants from radiation damage.
Amend soil with extremophilic microbes such as N_2-fixers

ALS 398 honors class

BO422 Plant Physiology class

Funded by **NIAC**

<u>Collaborators at NC State</u>

Nina S. Allen, Director of the Cell & Molecular Imaging Facility

Eva Johannes