Redesigning Living Organisms to Survive on Mars

Amy M. Grunden and Wendy F. Boss

Departments of Microbiology and Plant Biology

North Carolina State University

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

Working hypothesis:

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

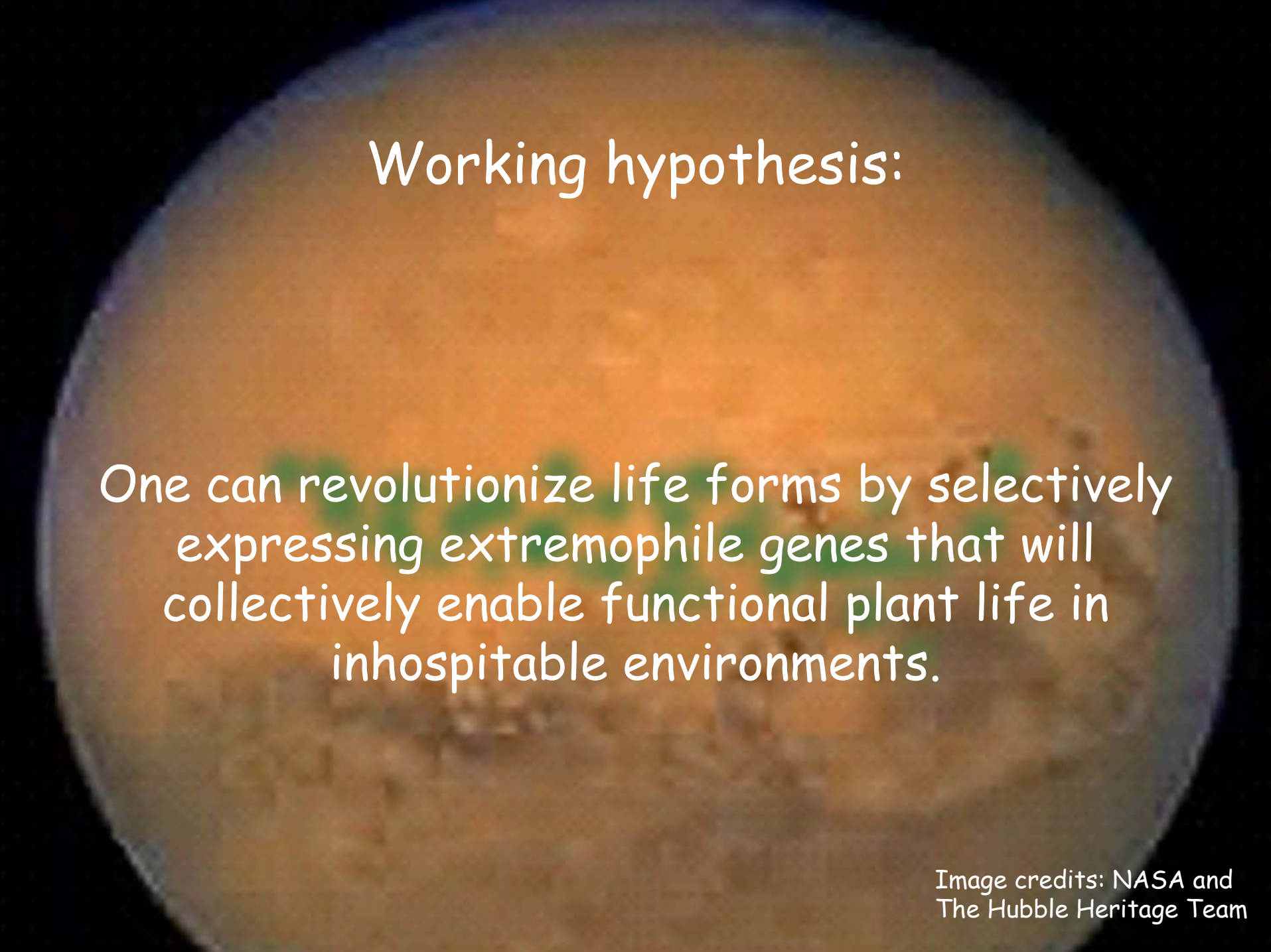


Image credits: NASA and The Hubble Heritage Team

Phase II Goals:

A. To use genes from the extremophile *Pyrococcus furiosus* to introduce new mechanisms to reduce toxic free radicals in plants

B. To use genes from the psychrophile, *Colwellia psychrerythraea* to develop new approaches to increase cold and dehydration tolerance

Image credits: NASA and The Hubble Heritage Team

Phase II Goals:

C. To produce hybrid plants and characterize their phenotype

D. To engage undergraduates in identifying additional mechanisms to resist radiation damage from radiation tolerant microbes

Image credits: NASA and The Hubble Heritage Team

Proof of concept (Phase I)

A. Produced a functional extremophilic protein in a model plant cell culture system

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

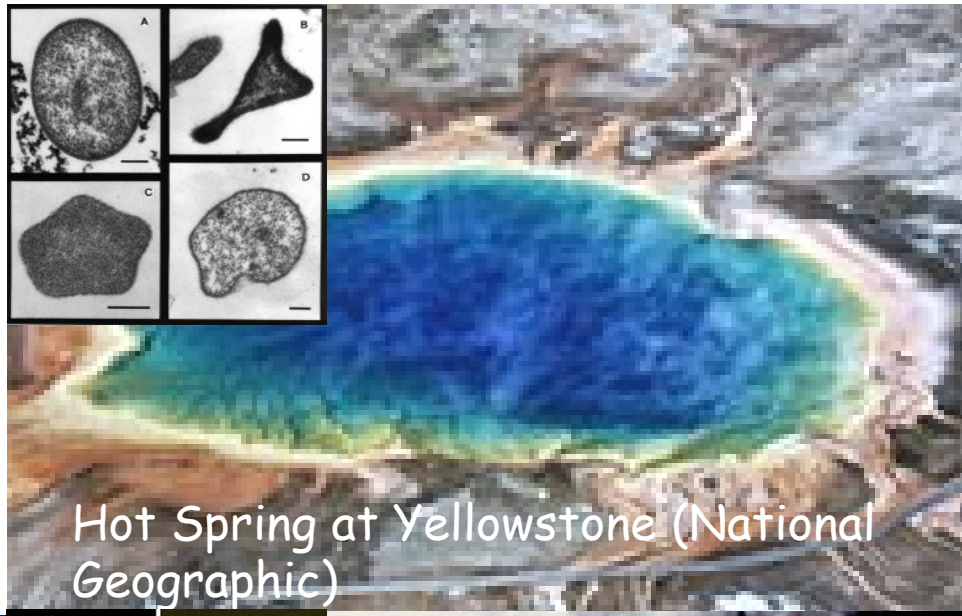
Assumptions and Challenges for Proof of Concept:

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

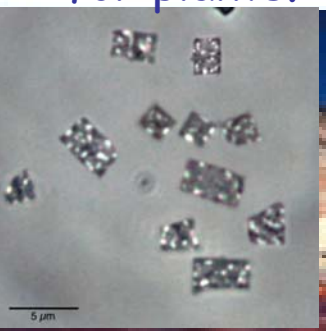
The functional archaeal protein would not harm the plant.

Why Extremophiles?

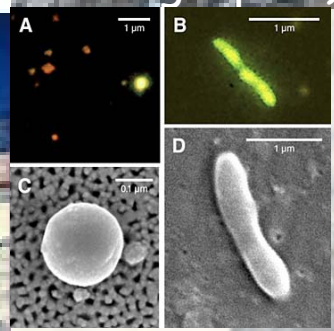
- The conditions on Mars are beyond what plants as we know them can survive and provide a bioregenerative life support system for human exploration.
- By combining strategies used by microorganisms which can survive and thrive in extreme environments we propose to make a major advance in extending the growth environment for plants.



Hot Spring at Yellowstone (National Geographic)

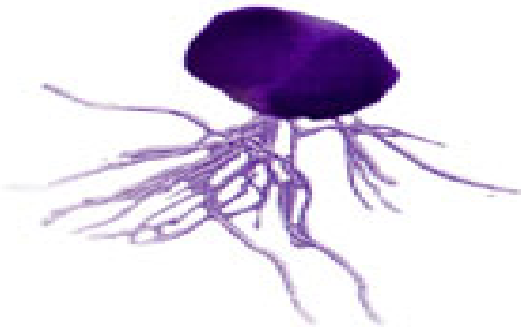


Owens Salt Lake (W. P. Armstrong)



Arctic Ice (T. Mock)

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.

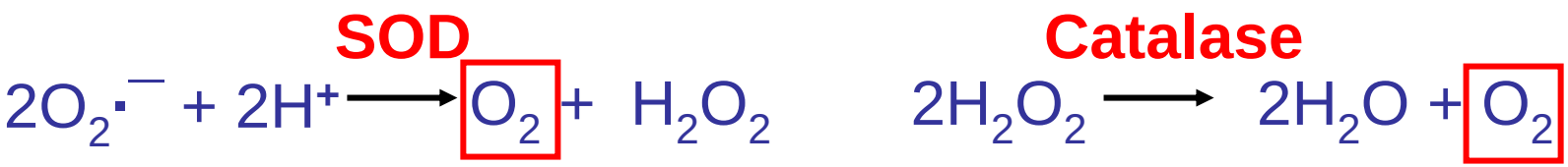
SOR is heat stable so that it can be assayed after heat denaturing the plant enzymes.

SOR is active from temperatures ranging from 0° to 100 °C.

In response to environmental stresses such as radiation, cold, heat, anoxia and drought, plants produce reactive oxygen species (ROS) such as superoxide and hydrogen peroxide.

While the initial increase in ROS can serve as a stress signal, a sustained increase in ROS will kill the plant.

To prevent the build up of excess ROS, plants, like all aerobic organisms, have ROS detoxifying enzymes such as superoxide dismutases (SOD), catalases and peroxidases.



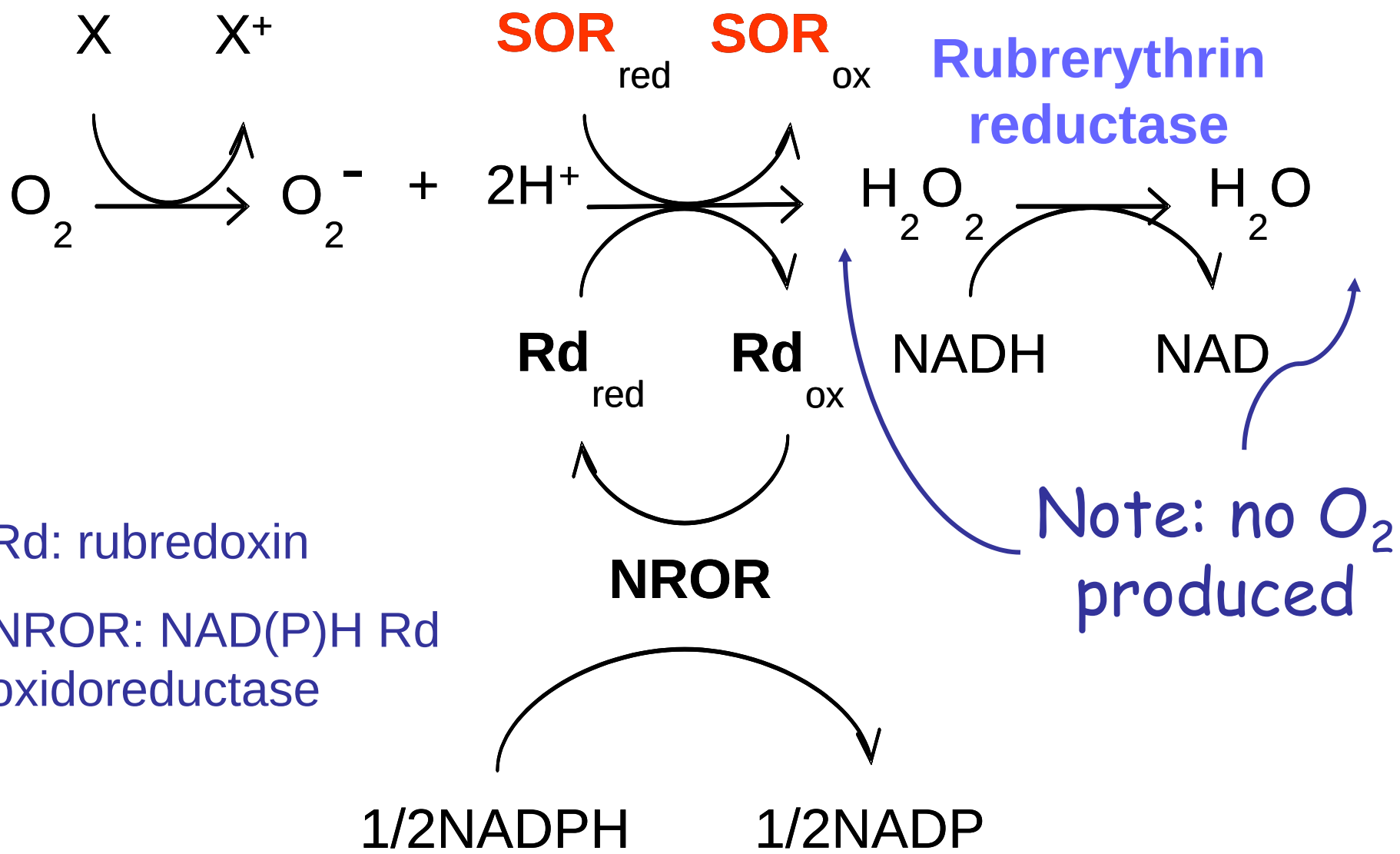
We propose to introduce a more efficient system to reduce ROS.

The initial ROS signal will occur but will not be sustained.

The byproduct, oxygen, will not be produced.

Our hypothesis is that this will decrease free radical propagation and ROS toxicity.

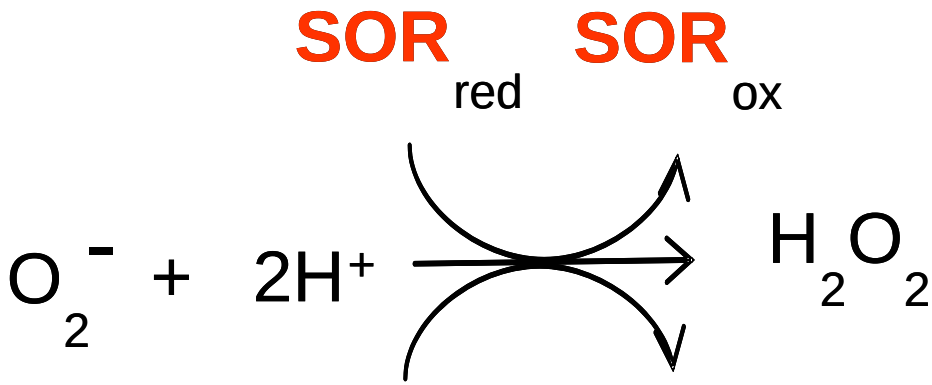
Superoxide Detoxification System in *Pyrococcus furiosus*



Rd: rubredoxin

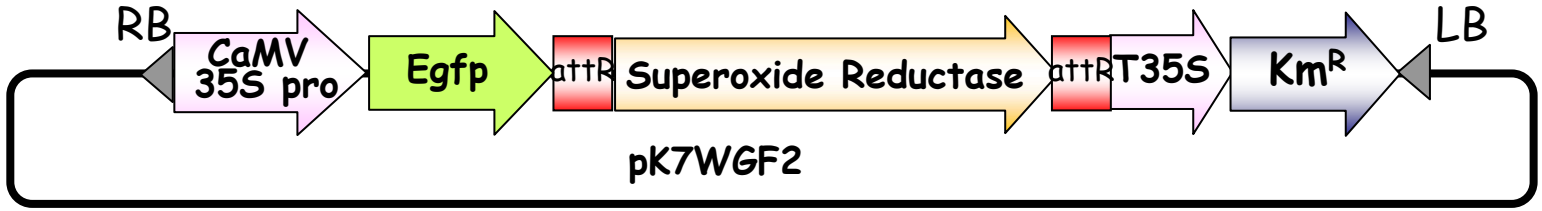
NROR: NAD(P)H Rd oxidoreductase

Proof of concept: Expression of *P. furiosus* superoxide reductase **(SOR) in plant systems**



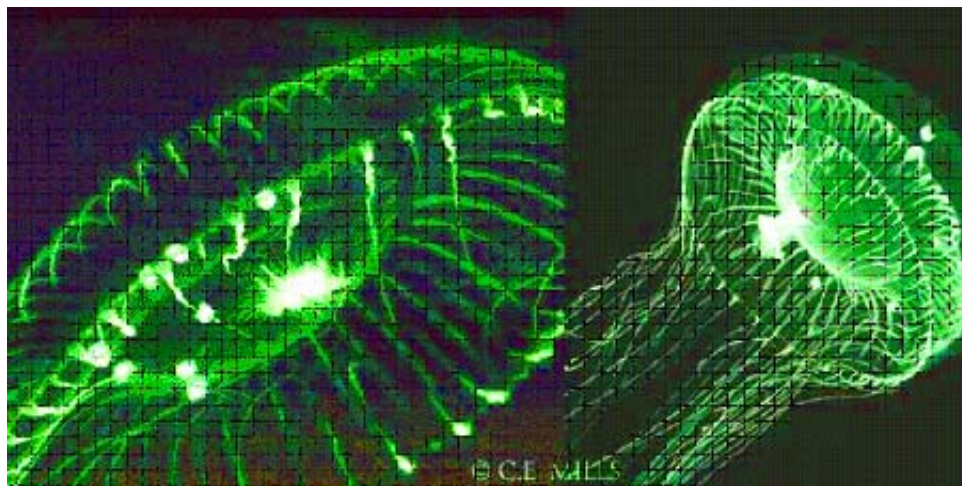
SOR will reduce superoxide but will increase cellular hydrogen peroxide. We anticipated that under normal conditions, the transgenic cells would have sufficient catalase activity to eliminate any excess hydrogen peroxide and that they would survive.

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



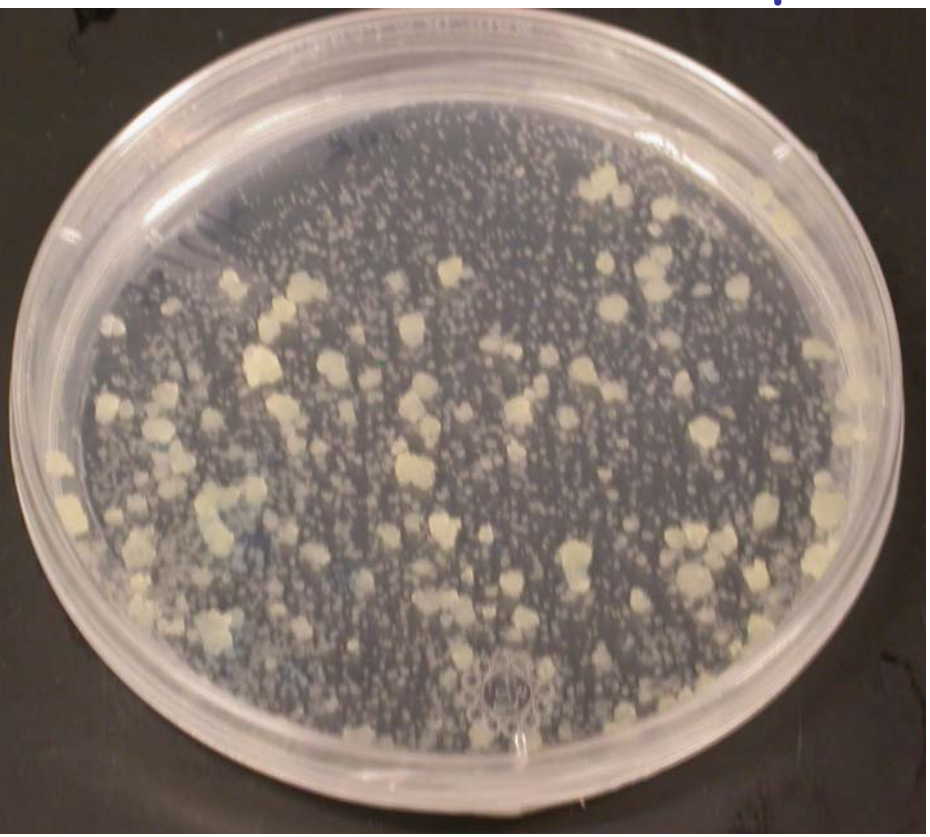
Provided by the Functional Genomics Division of the Department of Plant Systems Biology
http://www.psb.ugent.be/gateway/index.php

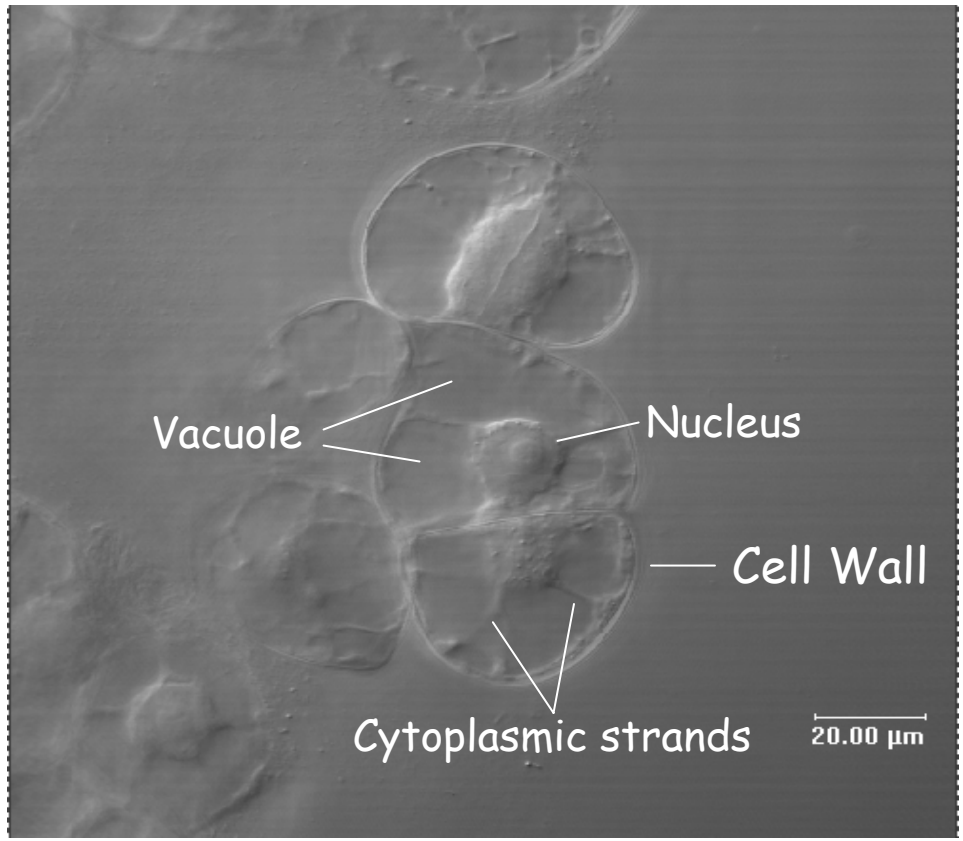
Green fluorescent protein from the Pacific jellyfish, *Aequoria victoria,* when fused to your favorite protein will reveal the subcellular location of the recombinant fusion protein.



Plant Cell Transformation

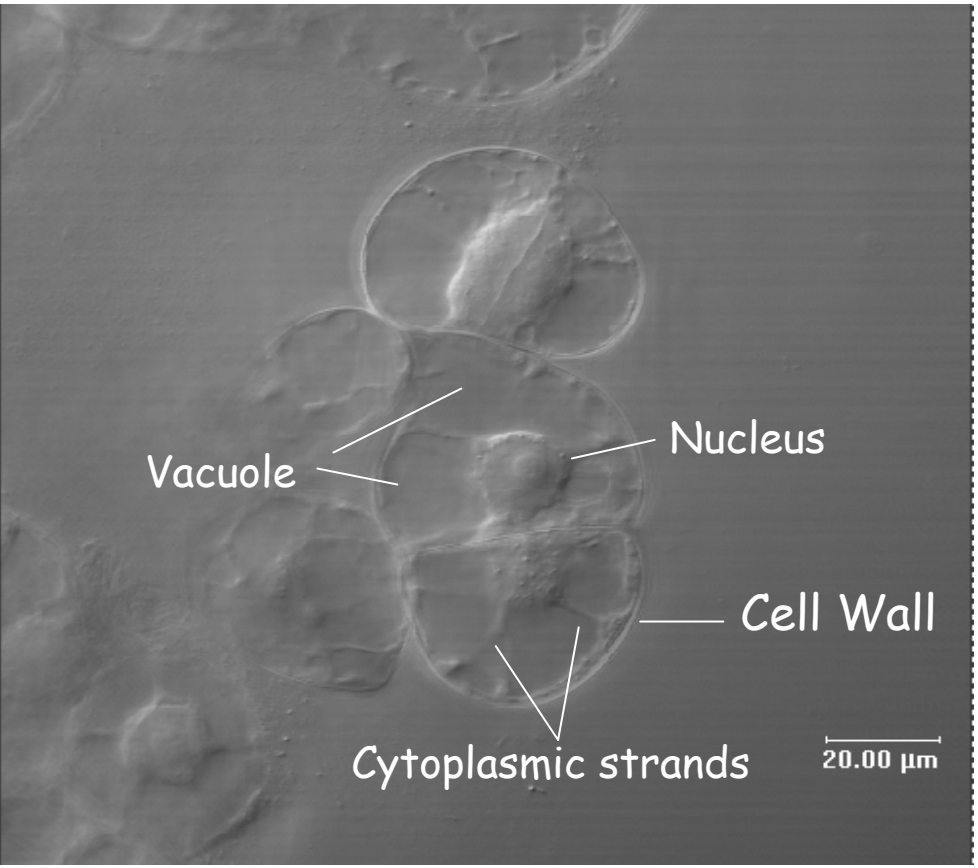
For proof of concept we used a relatively rapid plant transformation system: tobacco cells grown in suspension culture.

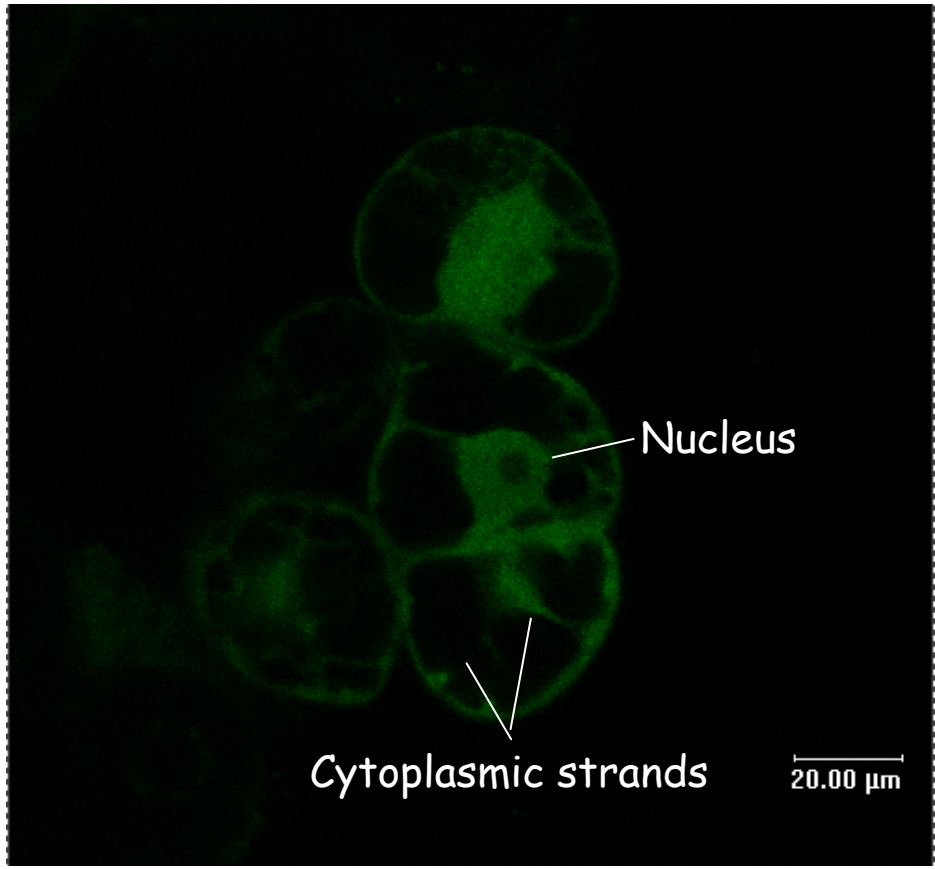


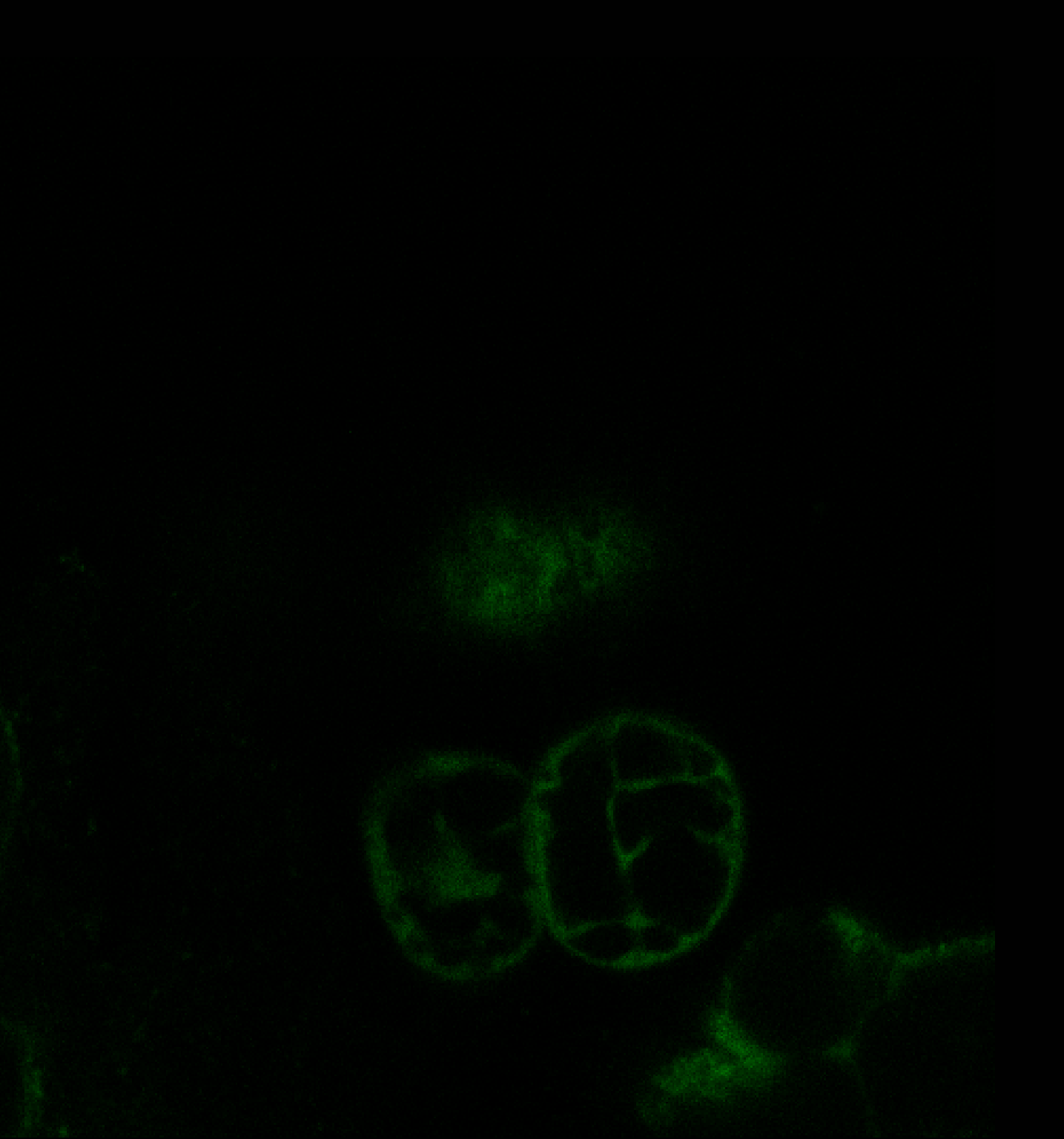


NT1 tobacco cell culture transformed with the Pf-SOR expression plasmid

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

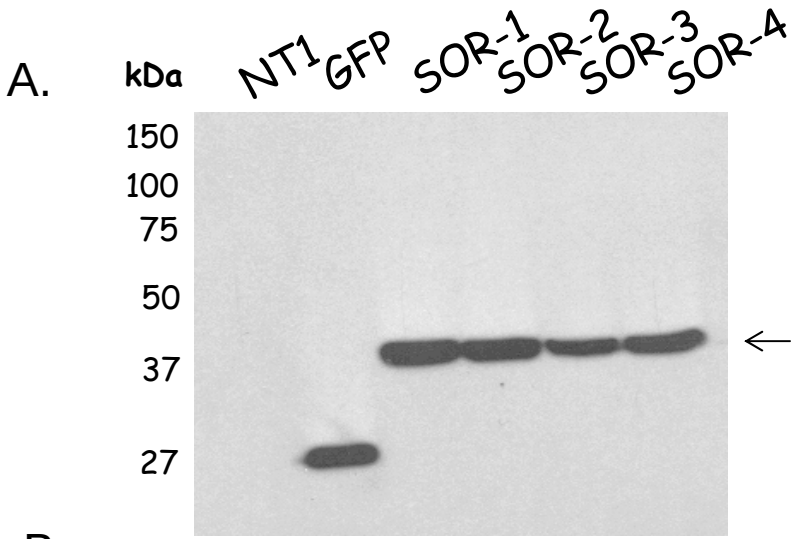




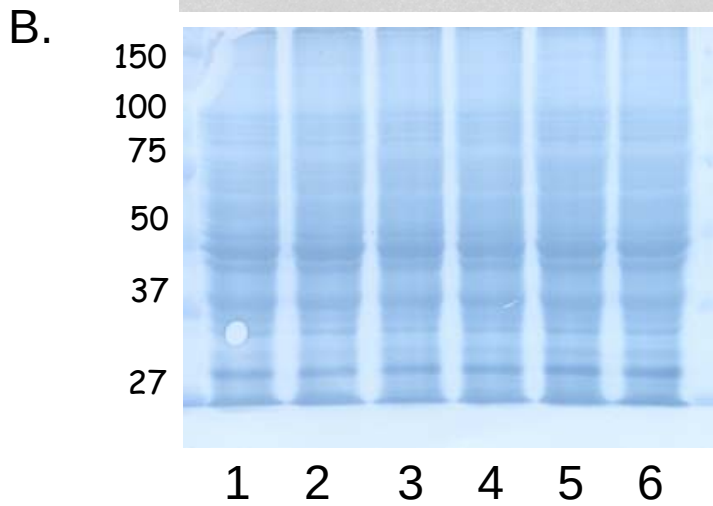


The full length GFP-SOR protein can be recovered from the transformed cells.

Immunoblot with GFP monoclonal antibody



Imido black Stained blot



NT1: Wild type NT-1 tobacco cells; GFP: Transgenic tobacco cells expressing GFP
SOR-1-4 : Transgenic tobacco cells expressing *P. furiosus* SOR

Recombinant *P. furiosus* SOR is functional and heat stable when produced in tobacco cell culture

Sample	Specific Activity (U/mg)	
	Untreated cell extract	Heat-treated cell extract
NT1 wild type	17.8 ± 0.4	6.9 ± 0.9
NT1-GFP	3.7 ± 0.2	1.4 ± 0.7
NT1-SOR1	28.3 ± 6.6	23.8 ± 3.5
NT1-SOR2	30.7 ± 12.3	19.6 ± 4.5
NT1-SOR3	37.9 ± 10.6	37.9 ± 5.6
NT1-SOR4	39.5 ± 4.3	42.1 ± 1.9

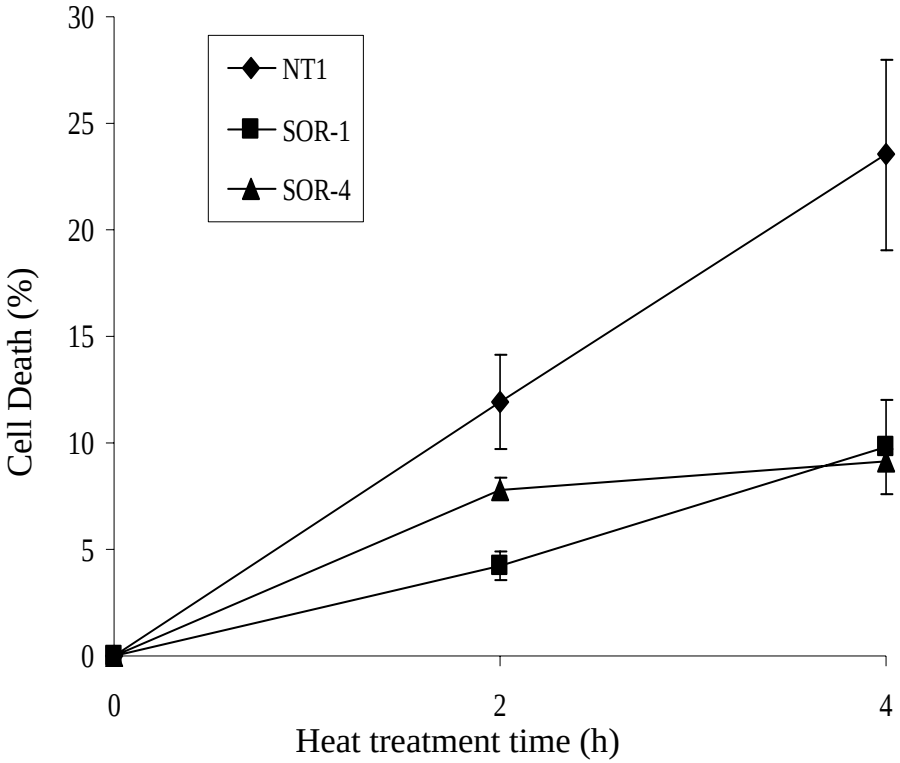
Im et al., FEBS Letters 2005 (in press)

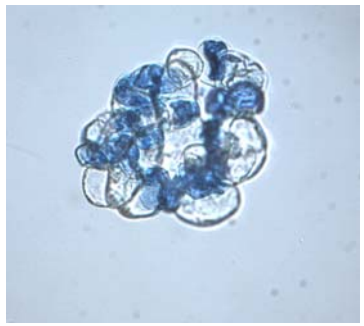
Reconstitution of heat-stable superoxide reductase activity from a transgenic tobacco cell culture strain

Sample	Specific Activity (U/mg)
NT1-GFP	1.6
NT1-GFP, HT	0.23
NT1-GFP, HT+Rd, NROR, NADPH	0.14
NT1-SOR3	17.2
NT1-SOR3, HT	0.12
NT1-SOR3, HT+Rd, NROR, NADPH	25.9

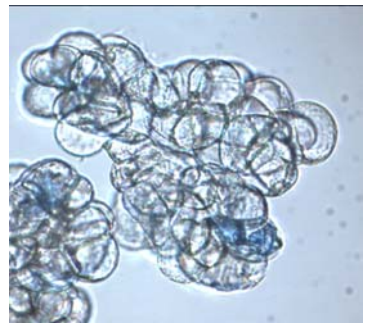
Im et al., FEBS Letters 2005 (in press)

Expression of SOR in tobacco cell culture confers an initial increase in heat tolerance.





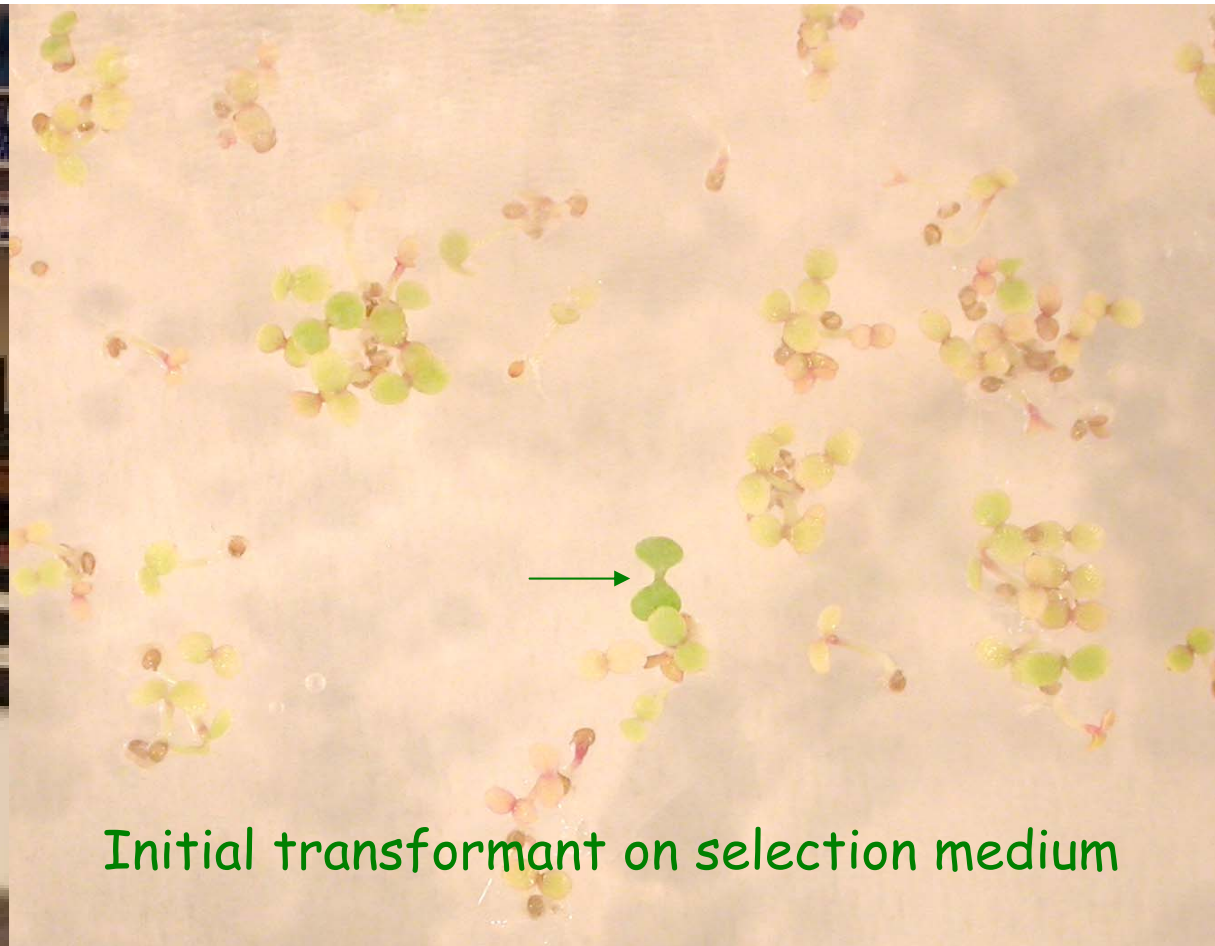
NT-1



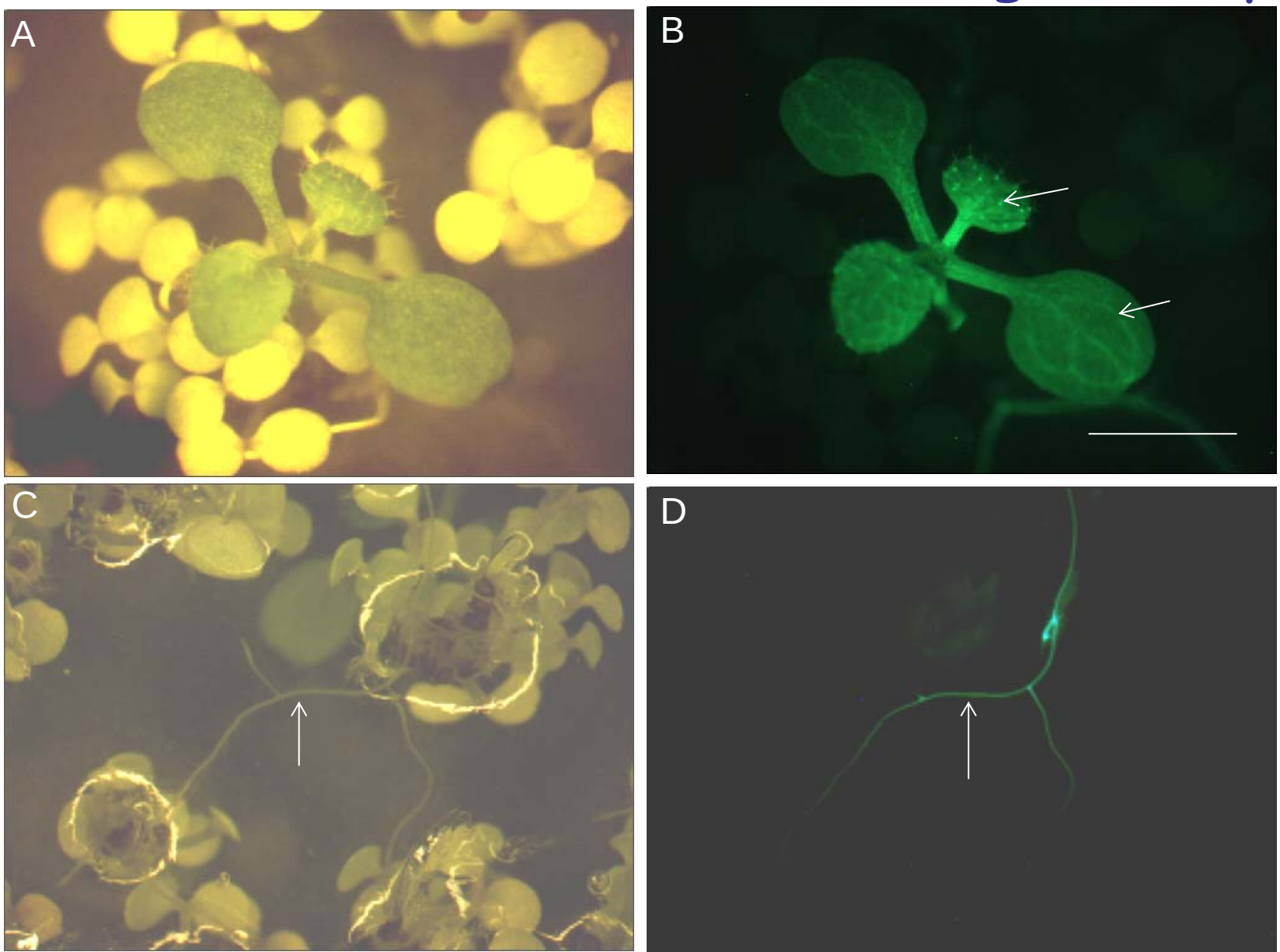
SOR-4

Im et al., FEBS Letters 2005 (in press).

The next step was to transform *Arabidopsis thaliana* plants with *P. furiosus* SOR and assess plant growth.

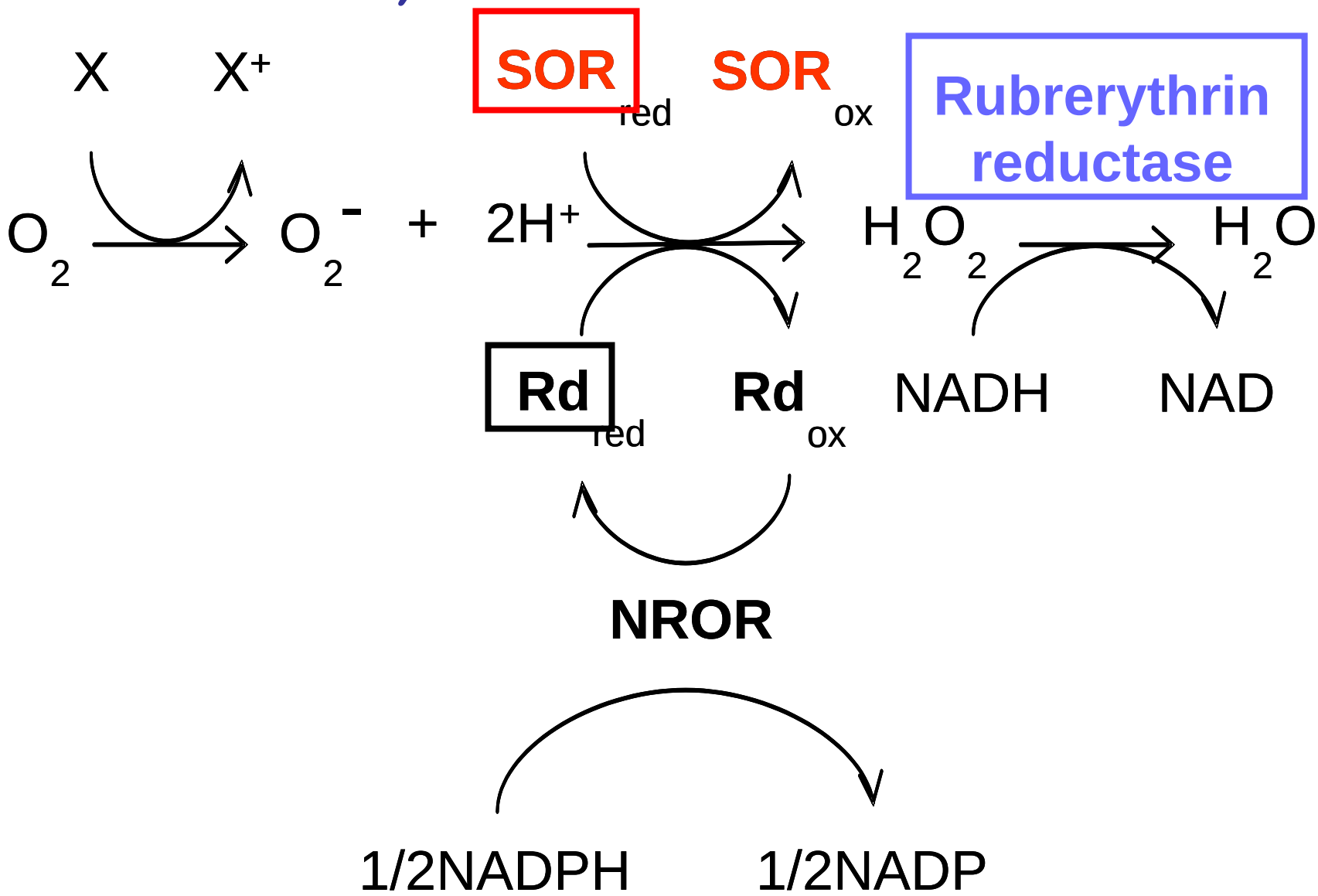


Arabidopsis plants expressing GFP-SOR are selected on medium containing kanamycin.

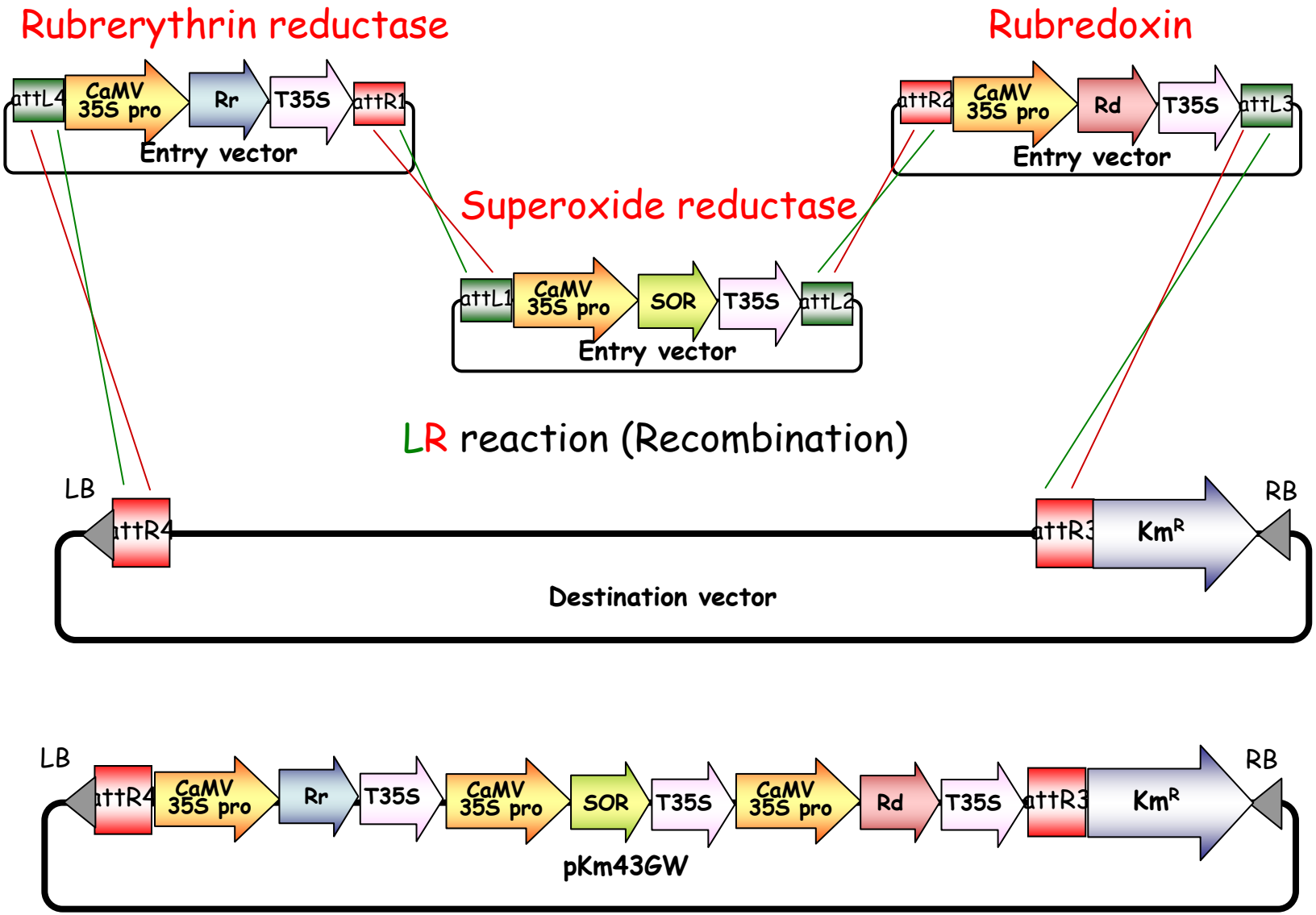


Now that we have shown we can produce functional *Pyrococcus furiosus* proteins in plants, we propose to include the other enzymes in the pathway to reduce hydrogen peroxide as well as superoxide production.

Superoxide Detoxification System in *Pyrococcus furiosus*



Progress towards Phase II goals: Designed vector for multiple gene expression



Select genes from the psychrophile, *Colwellia psychrerythraea* to enhance cold and drought tolerance.

The availability of reduced glutathione can mean the difference in survival or death during and after exposure to cold stress.

Reduced glutathione (GSH) will reversibly complex with proteins, stabilize their structure and prevent denaturing.

GSH + Protein-SH ⟶ Protein-SG (stable)

Glutathione Reductase

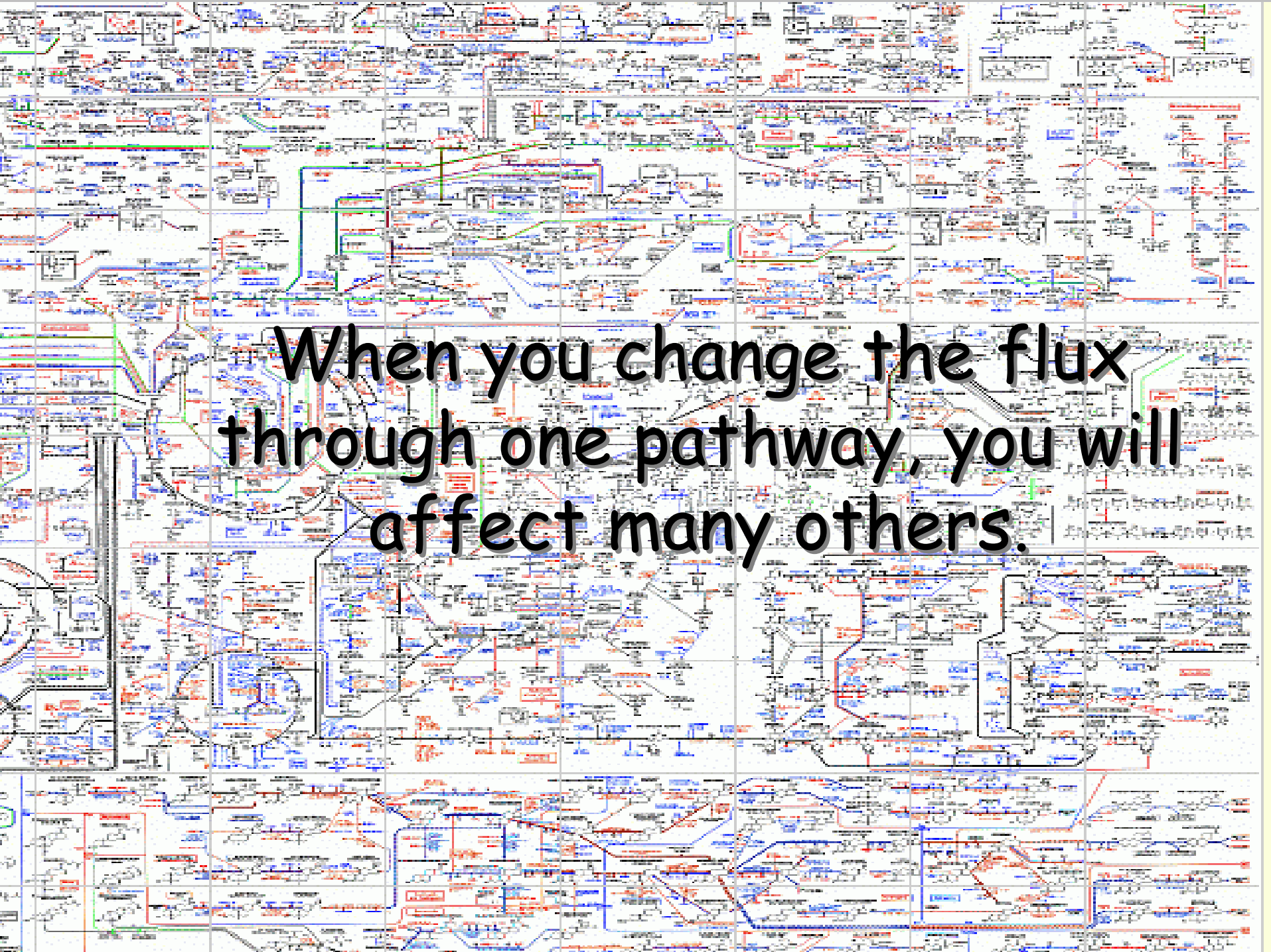
GSSG ⟶⟵ GSH (reduced glutathione)

Our hypothesis is that increasing glutathione reductase will increase the pool of reduced glutathione available to complex with and stabilize proteins during rapid temperature fluctuations.

Glutathione reductase from the psychrophile, *Colwellia psychrerythraea,* will function at the growth temperatures of the organism (0° to 20 °C).

Challenges for Phase II Studies:

- To have all three genes encoding SOR pathway enzymes expressed.
- To have the functional enzymes localized in the optimal region of the cell.
- To have the SOR pathway enzymes and glutathione reductase function in plants without deleterious effects on the physiology of the plant.


When you change the flux through one pathway, you will affect many others.

- We will assess the effects of altering ROS accumulation and the glutathionylation of proteins in a model system in order to use the databases available to analyze the impact on plant metabolism and physiology.

- Use these insights to work with NASA scientists and breeders to add extremophile survival traits to plants selected for space flight.

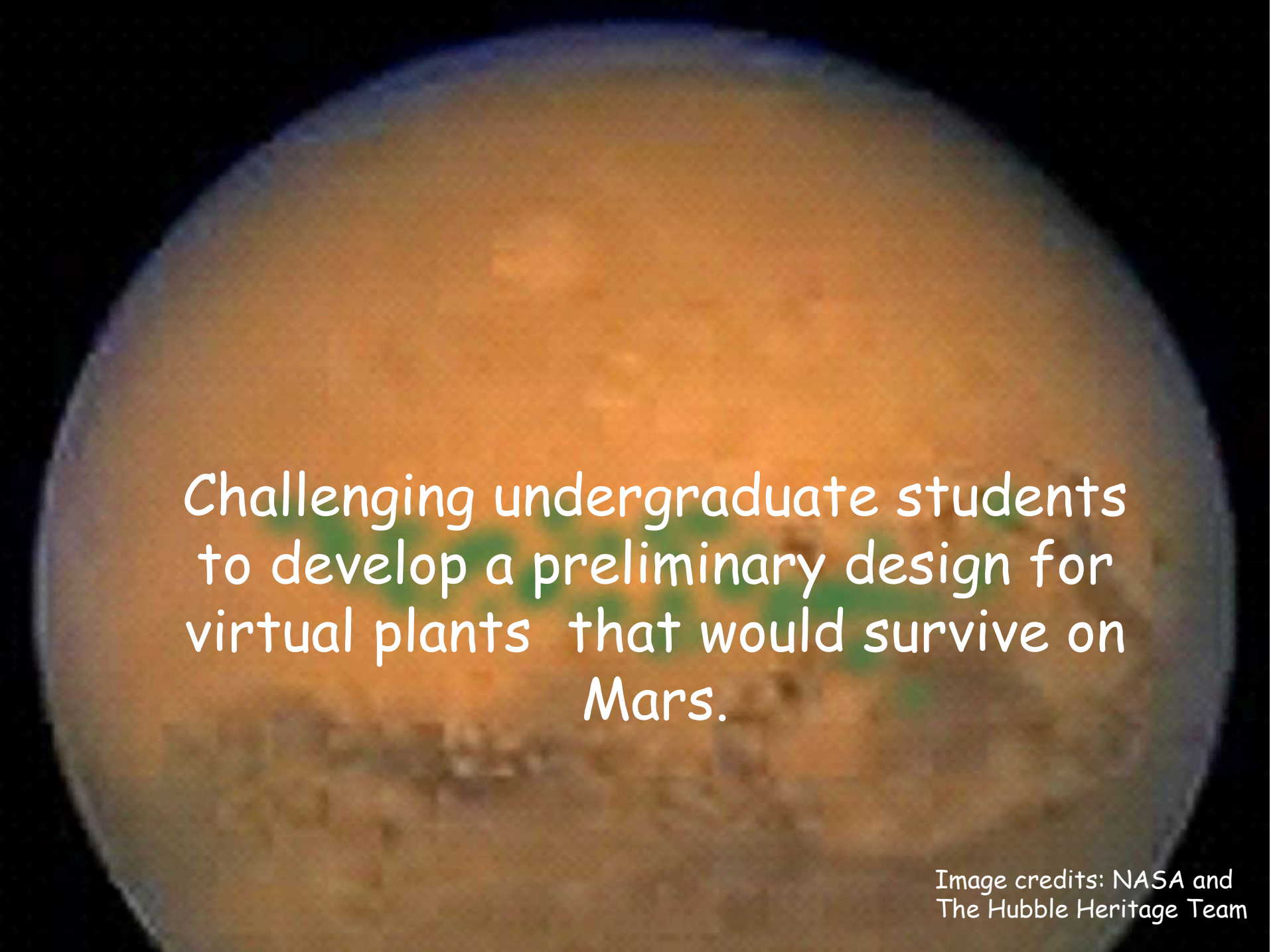


Image credits: NASA and The Hubble Heritage Team

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

- Developed an Honors class: "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 presented their designs during a mock press conference on April 26, 2005 and as a written research paper

Student Project Groups

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

- Students presented their project in a press conference format

Honors students sharing their concepts for "Redesigning Plants to Survive on Mars" during their oral presentation on April 26, 2005

Spring of 2007
Honors Class

"Identifying radiation resistance mechanisms from radiation tolerant microbes to introduce into plant systems "

Phase II Redesigning Life for Survival on Mars Road-map

- Produce transgenic plants that express cold-active carbon metabolism and photosynthesis genes to support **robust** growth at cold temperatures (10 years)
- Enhance plants' capacities to resist radiation damage by expressing DNA repair genes from radiation resistant microbes (10-15 years)
- Enhance plants capacity to resist ROS stress under extreme environmental conditions (Phase I and phase II, 3 years)

ALS 398 honors class

BO422 Plant Physiology class

Funded by **NIAC**

Collaborators at NC State

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

Eva Johannes