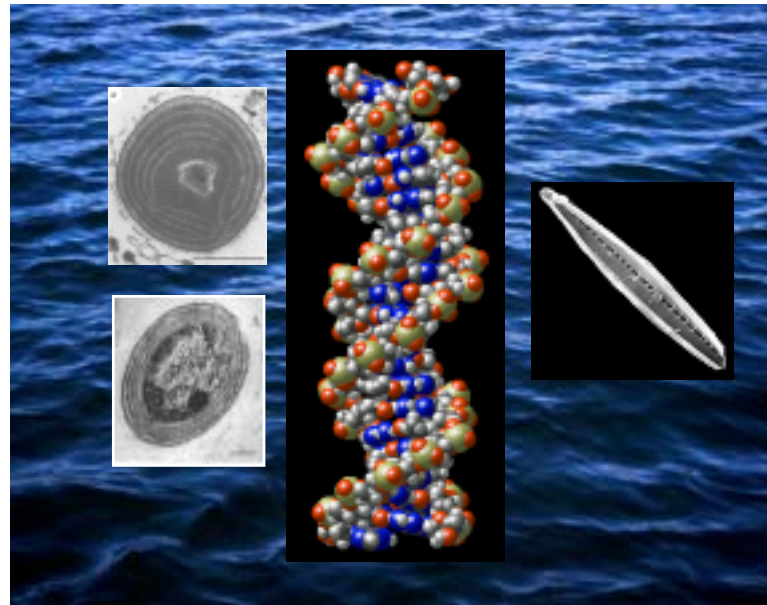


MARGENES

Marine phytoplankton as novel model organisms for genomic and post-genomic studies of environmental sensing and niche adaptation

<http://www2.warwick.ac.uk/fac/sci/bio/research/margenes>



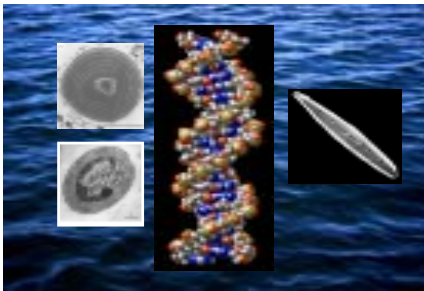
***Synechococcus* WH8102**
***Prochlorococcus* PCC9511**
Phaeodactylum tricornutum

**Model photosynthetic organisms for studying
genes implicated in**

Environmental sensing

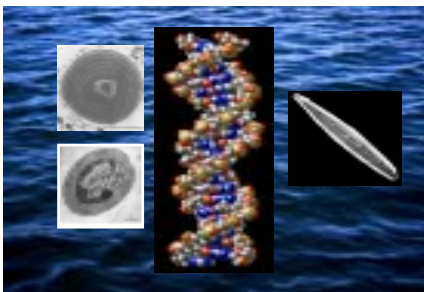
Stress response

Niche adaptation



Objectives

- To develop (or improve existing) mutagenesis/transformation systems, expression systems and DNA arrays for marine cyanobacteria and one marine diatom
- Assess how these organisms perceive their environment, acquire light and nutrients and respond to environmental stresses
- Study the role and regulation of those genes implicated in the adaptation to specific niches in the ocean
- Compare strategies developed by prokaryotes and eukaryotes to deal with environmental sensing and niche adaptation



Structure:

WP1
Genomics

WP2
Genetics

WP3
Light
Photoprotection
Acquisition
Stress

WP4
Nutrients
Perception
Acquisition
Stress

Partners:

D. Scanlan
Warwick
Co-ordinator

F. Partensky
D. Vaultot
Roscoff

W. Hess
Berlin

A. Post
Eilat

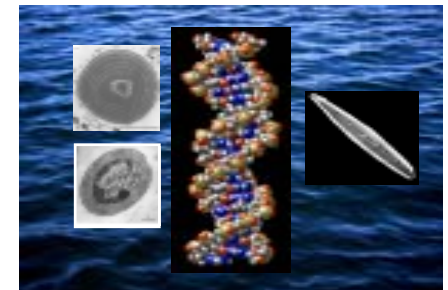
C. Bowler
Naples

P. Kroth
Konstanz

P. Lopez
Paris

Expected Outcomes:

Understanding gene function of some key representatives of phytoplankton communities will help decipher the processes by which marine species manage to adapt to their specific habitat and ultimately predict the short- and long-term behaviour of such organisms in a changing environment



Marine cyanobacteria :

Prochlorococcus and *Synechococcus* – represent ecologically relevant components of the phytoplankton.

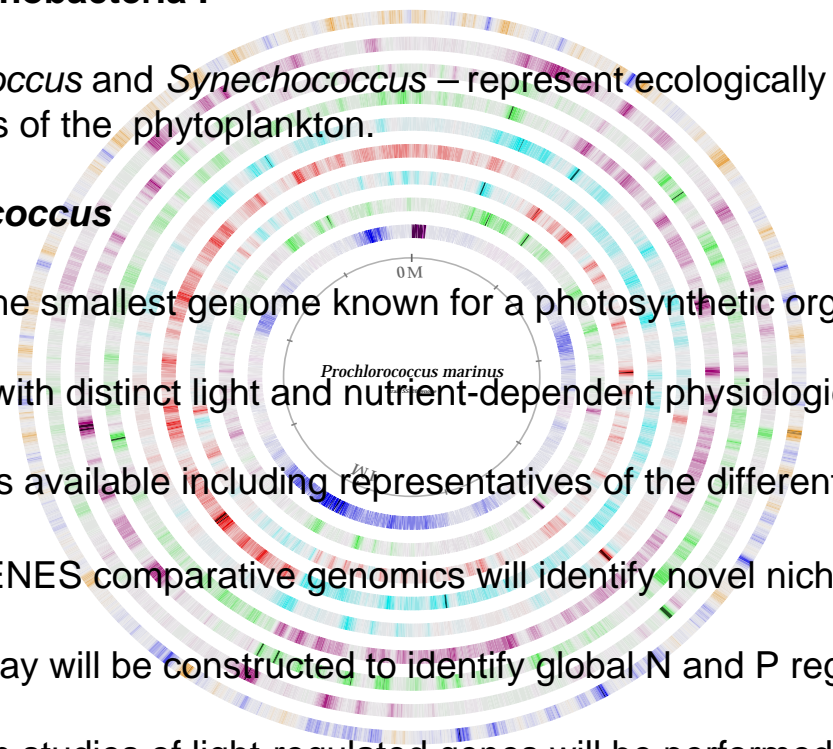
Prochlorococcus

- contains the smallest genome known for a photosynthetic organism
- ecotypes with distinct light and nutrient-dependent physiologies exist
- 3 genomes available including representatives of the different ecotypes

In MARGENES comparative genomics will identify novel niche-specific genes

A microarray will be constructed to identify global N and P regulons

Expression studies of light-regulated genes will be performed



<http://www.cbs.dtu.dk/>
Center for Biological Sequence Analysis
Prochlorococcus marinus



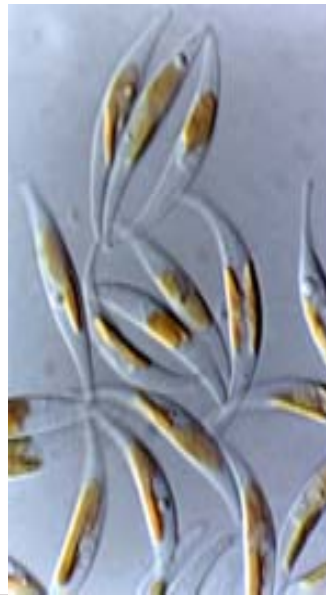
Synechococcus WH 8102

Marine Synechococcus

- several distinct phylogenetic clades can be distinguished
- strains representative of specific clades have been isolated
- can easily be grown on plates and a genetic system exists
- complete genome of *Synechococcus* sp. WH8102 available
- In MARGENES the marine *Synechococcus* system, which allows the easy construction of mutant strains, will be used to determine the function of genes involved in regulation of nutrient transport and photoprotection



- Searchable EST database now available



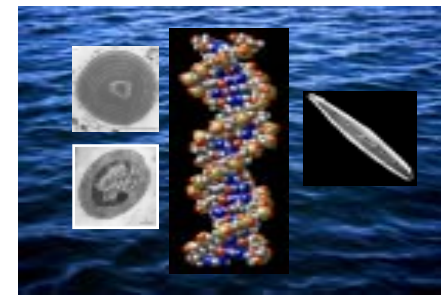
***Phaeodactylum tricornutum*: a model diatom species for functional genomics**

- EST database now available
- Easily transformable: reporter genes, over-expression cassettes, RNAi technology all available
- Well studied physiology
- Sexual crosses can be performed under laboratory conditions
- Small genome (15 Mb) with GC content of 48.5% that is not highly methylated
- Will allow centric:pennate comparisons

MARGENES will :

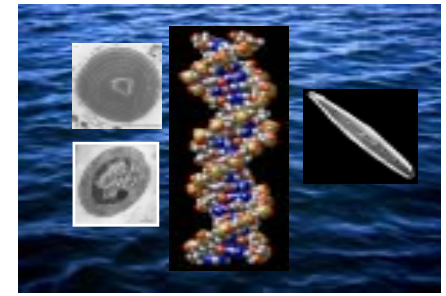
- enlarge the *Phaeodactylum* EST database,
- investigate *Phaeodactylum* photoperceptory processes,
- identify nutrient responsive genes and
- further expand genetic manipulation systems for this organism

Overview



Workpackage	Tasks
WP1	<ol style="list-style-type: none">1.1. Annotation of the <i>Prochlorococcus marinus</i> SS120 genome1.2. Increasing the size of the <i>Phaeodactylum</i> EST library and annotating ESTs1.3. Development of large scale expression analysis systems (DNA arrays) in marine phytoplankton
WP2	<ol style="list-style-type: none">2.1. Development of targeted mutagenesis or transformation systems in marine phytoplankton2.2. Development or improvement of expression systems in marine phytoplankton
WP3	<ol style="list-style-type: none">3.1. Expression analysis of genes implicated in light perception, photoprotection, photosynthesis and general stress response in marine phytoplankton3.2. Targeted mutagenesis or transformation studies of selected genes implicated in light perception, photoprotection and/or general stress response in marine phytoplankton3.3. Over-expression studies of selected genes implicated in light perception in marine cyanobacteria
WP 4	<ol style="list-style-type: none">4.1. DNA array studies of the expression of genes implicated in nutrient perception and acquisition and nutrient stress response in marine cyanobacteria4.2. Mutagenesis of genes implicated in nutrient perception and acquisition and nutrient stress response in marine phytoplankton4.3. Iron and silica sensing and uptake in diatoms

Specific Aims (Partner 1)



1. P and nutrient transporters

Annotation of *Prochlorococcus* SS120 genome

Comparative genomics of marine and aquatic cyanobacteria

2. Improvements to genetics system for WH8102

3. Examination of the role of P sensing and acquisition genes in *Synechococcus* WH8102

Construction of k/o mutants eg. *ptrA*, *phoB/R*

Examination of phenotype

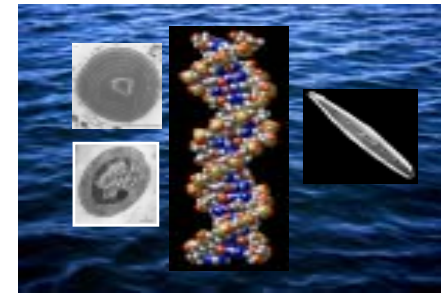
DNA Macro/Micro-Arrays

Proteomics of subcellular fractions

4. Investigate the role of any other genes identified in 3 as constituting the Pho regulon

Annotation and Comparative genomics

Partners 1-4



Annotation of the *Prochlorococcus marinus* SS120 genome

- automated draft annotation
- confirm orf designations

Manual editing

- Gene presence/absence
- Reconstruction of biochemical pathways
- Search for N-terminal fusions, frameshifts, alterations to important sites or amino acids

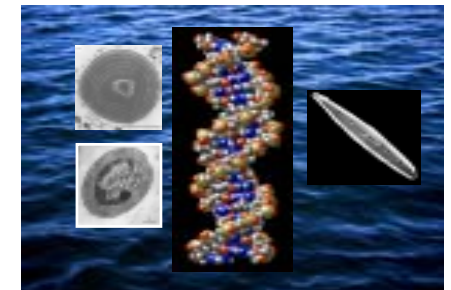
Comparative genomics (with *Prochlorococcus* sp. MED4, *Prochlorococcus* sp. MIT9313, & *Synechococcus* sp. WH8102)

Comparison of genomic regions

- Phylogeny
- Correlation with ecological data to identify potential niche specific genes

Improving *Synechococcus* sp. WH8102 genetics

- plating efficiency of WH8102
 - Media additions : antioxidants, LMP agarose, spent media, cell extracts, vitamins, mixed amino acids
 - Light regimes, filters
- Conjugations
 - Tri- vs Di(2)-parental
 - Helper donor plasmid range
 - Antibiotic range, MICs, methods of selection
 - Selection from liquid rather than solid media
- Gene expression
 - Use of reporter fusions and FACS



Analysis of P mutant (*ptrA/phoBR*) phenotypes

- Physiological response to P-limitation
 - Growth kinetics, Survival, Pigments profiles, Elemental composition, different sources of P, PstS, enzyme activities e.g. alkaline phosphatase
- P uptake kinetics
- Molecular response to P-limitation
 - DNA Macro/Micro Arrays
 - Proteomics

Outcomes

- Integrated understanding of the P sensing and acquisition machinery and niche adaptation
- Development of molecular probes to assess nutrient status *in situ*
- Novel mechanisms of adaptation to low nutrient environments ?

Other methods

Search for Pho box promoters in silico

