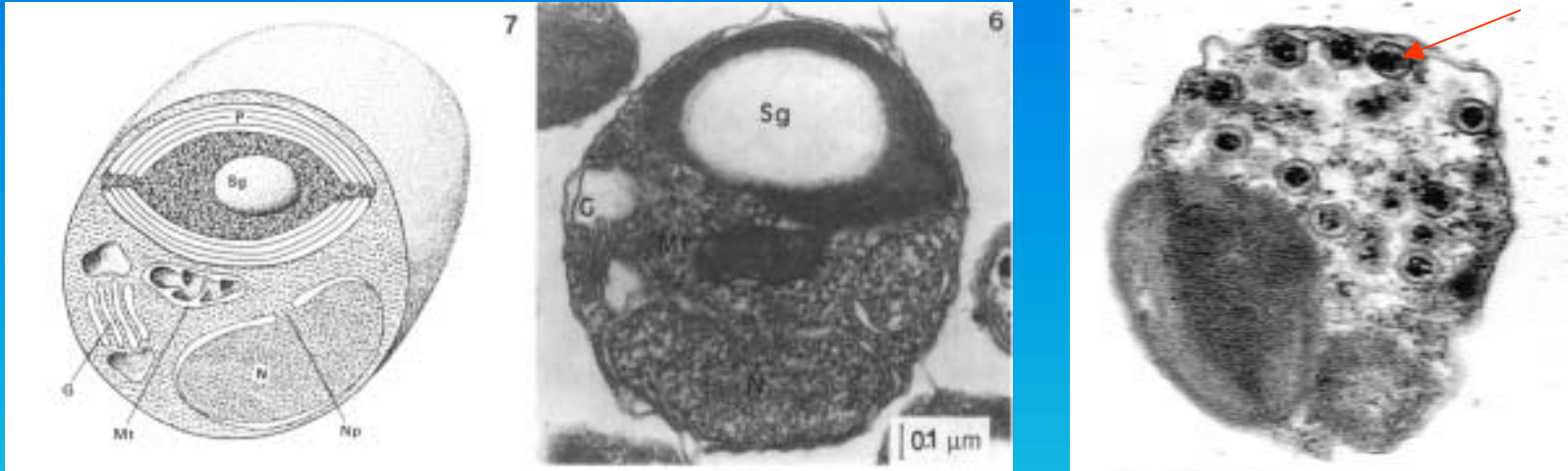


***Ostreococcus* :**

The smallest eukaryote as a model for cell biology

- **Introduction to *Ostreococcus tauri***
- **Genomics**
- **Cell cycle studies**

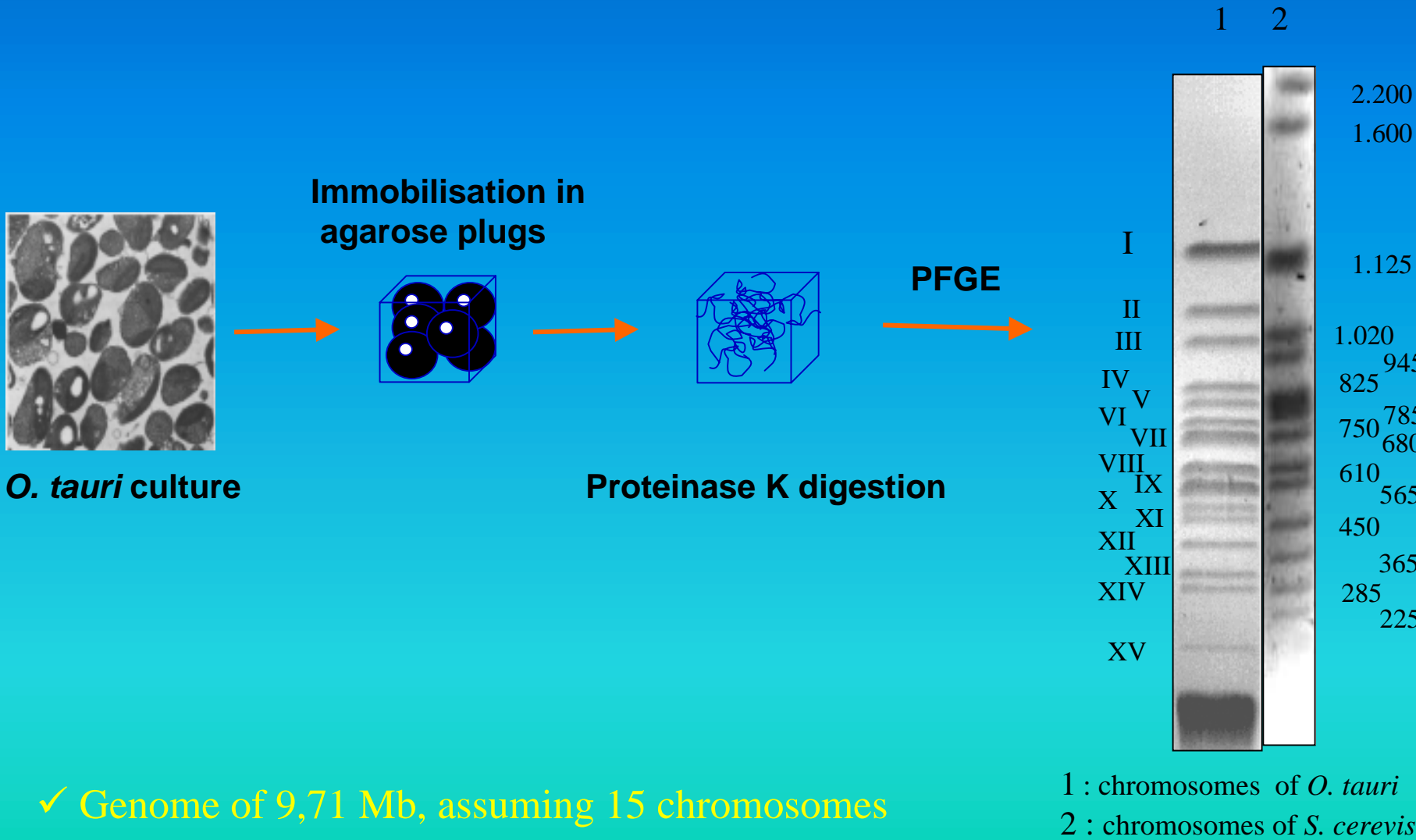
Ostreococcus tauri



From Chrétiennot-Dinet et al. (1995)

- Marine photosynthetic pico-eukaryote
- Isolated in Thau lagoon
- Observed by FCM - major fraction of the phytoplankton
- One of the smallest eukaryotes: 0.5-1 μm
- Minimal cellular organization
- Infected by a virus

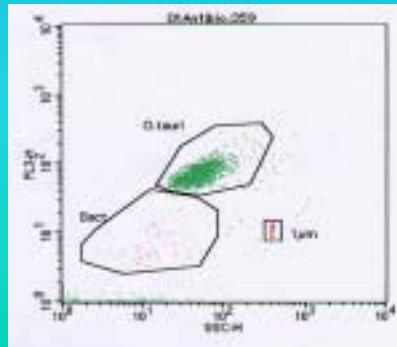
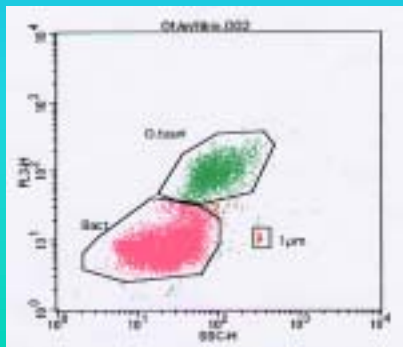
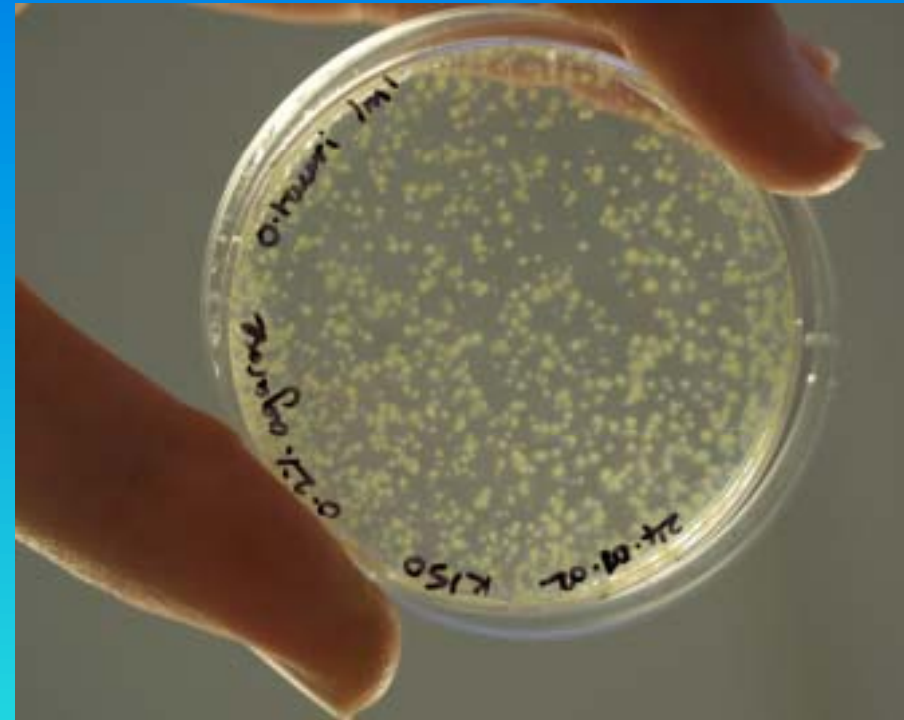
O. tauri karyotype



✓ Genome of 9,71 Mb, assuming 15 chromosomes

Culturing of *O. tauri*

- Culturable in media based on artificial sea water
- Fast growing - up to 3/4 divisions per day
- A 'pure' culture obtained after antibiotic treatment

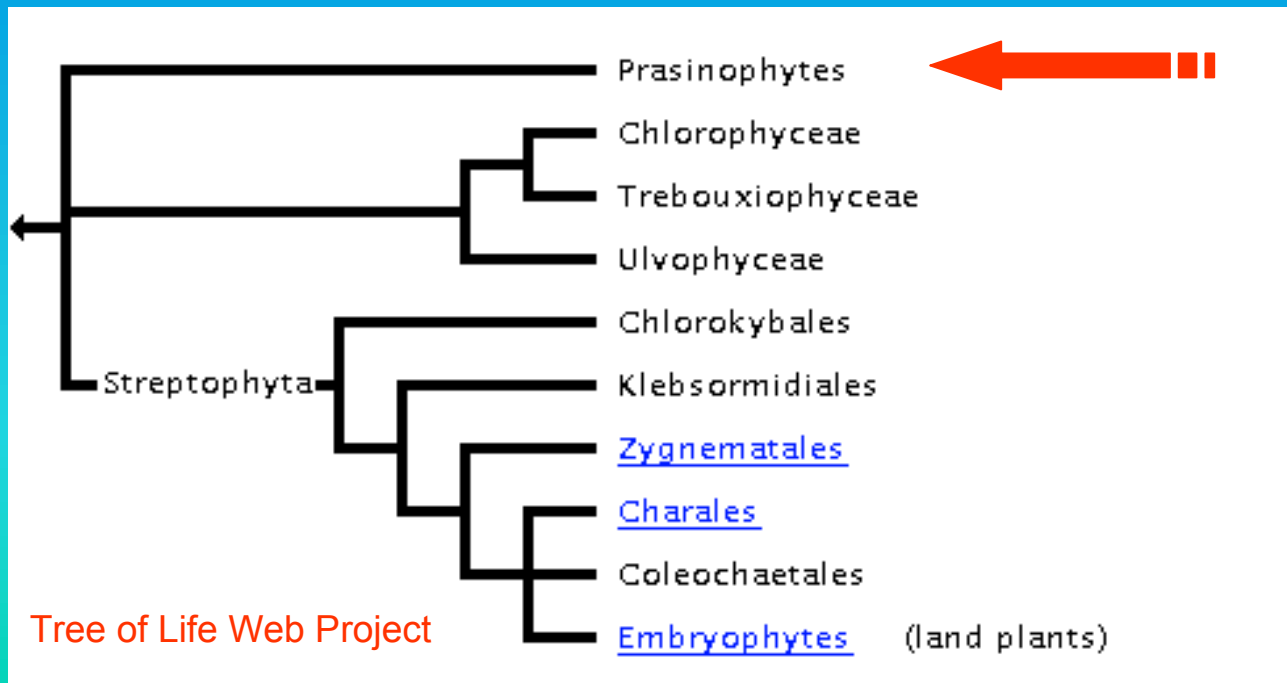


- Can be grown as single colonies in semi-solid agarose

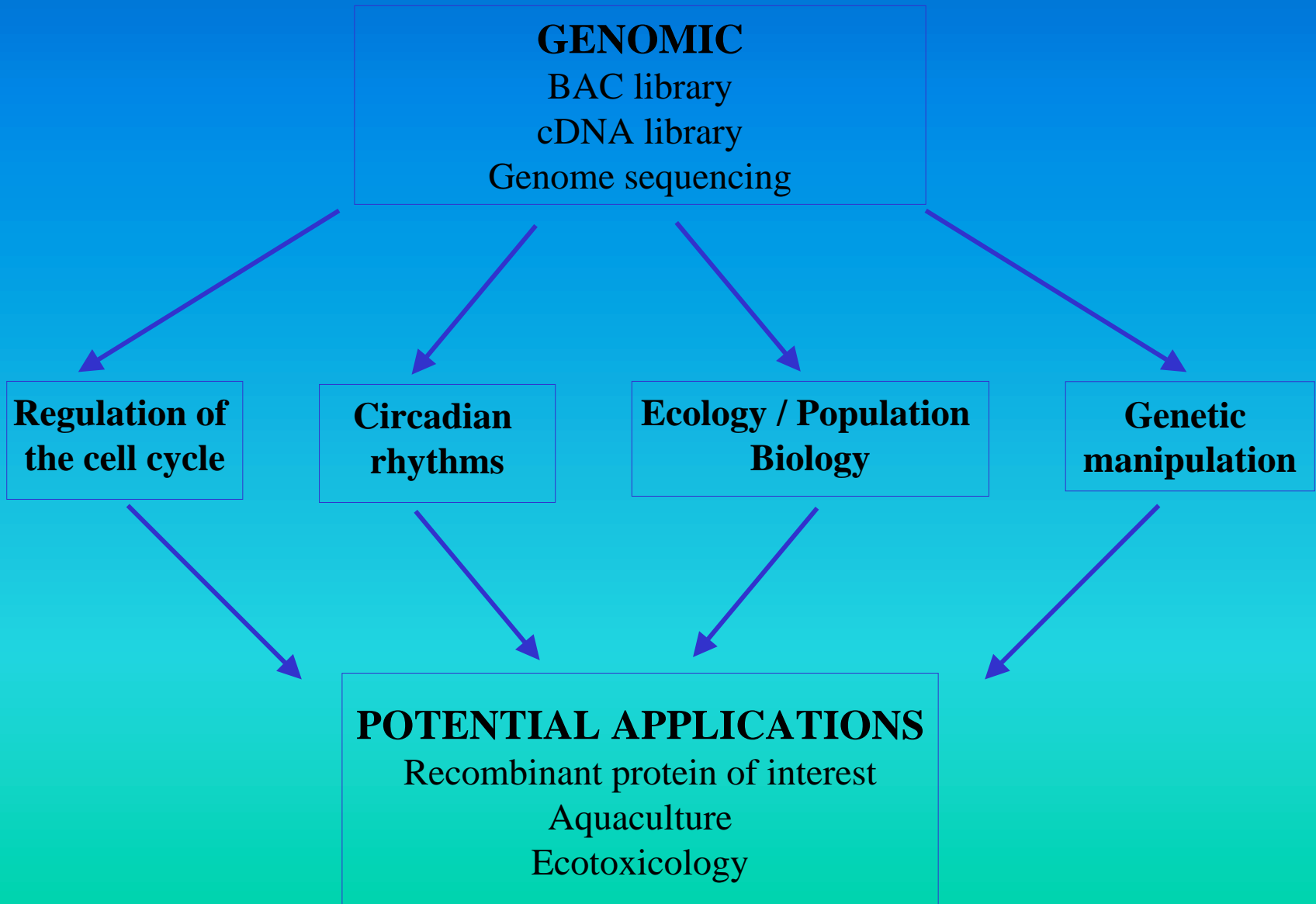
Phylogenetic position of *O. tauri*

- Prasinophyte
- Diverged at the base of the green plant lineage

Green plant lineage



Research themes



Genomics approach

Shotgun library

- Whole genome sequencing

BAC library

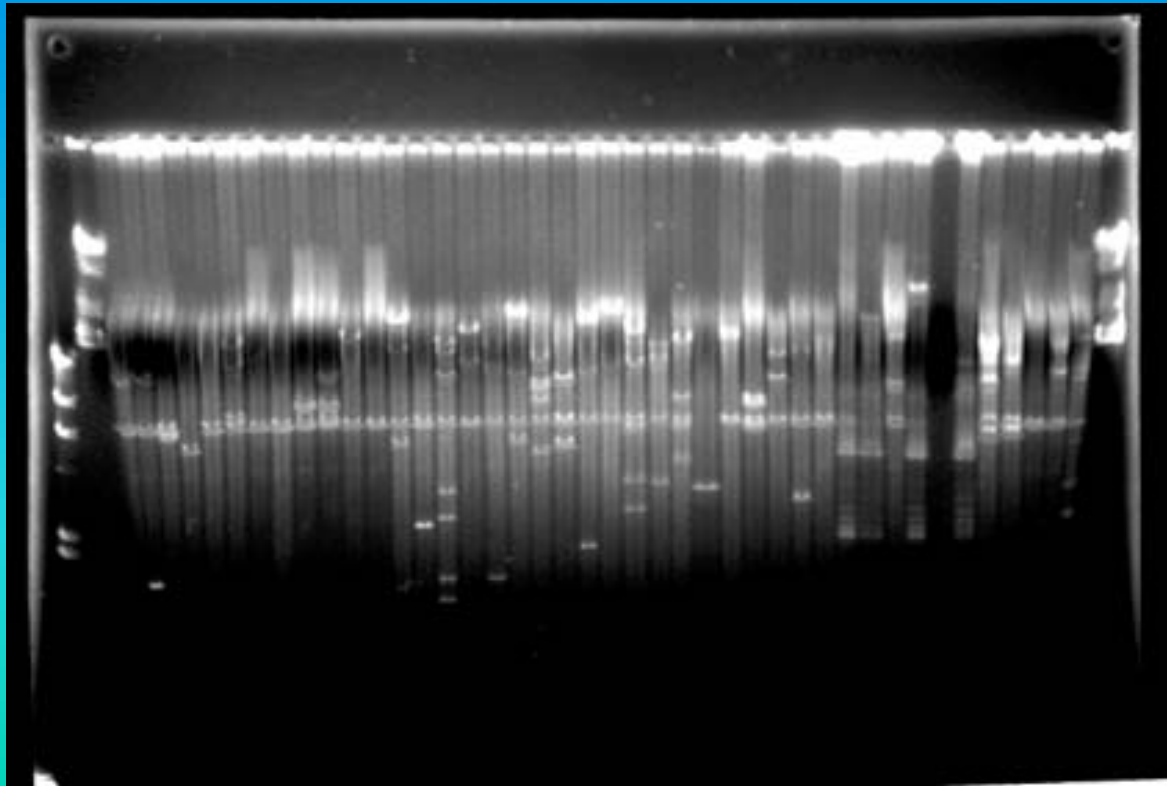
- Find gene of interest
- Complementary sequencing
- Genome annotation

cDNA library

- Find protein sequences → functional approach

Characterisation of the BAC library

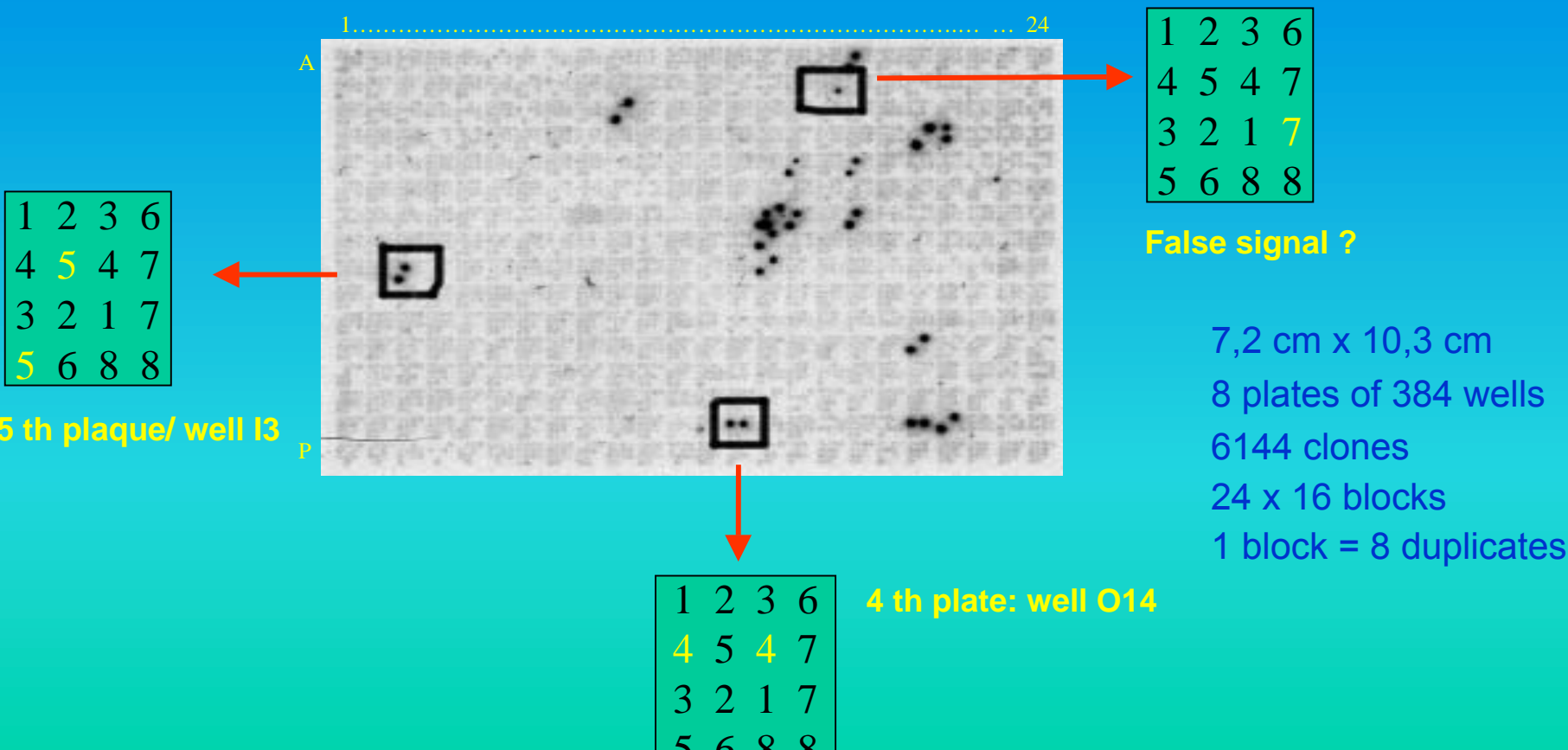
- 2457 clones distributed in 7 microplates
- Average size of inserts: 70-80 kb
- Theoretical coverage of the library: 15X



PFGE (3h30) of 44 minipreps digested with Not I

Replication of the library by robot

- Master microplates of 384 wells replicated
- Clones from replica microplates spotted onto high density nylon membranes
- Each membrane/macroarray contains the whole BAC library



Genome sequencing project

Genopole Languedoc-Roussillon

Scientific coordinator

- **Hervé Moreau**

Partners

- **Laboratoire Arago:** A. Picard, E. Derelle, H. Moreau
Preparation of DNA, BAC library and preliminary annotation of genome
- **University of Perpignan – IRD:** M. Delseny, R. Cooke, C. Berger et M. Laudié BAC end sequencing
- **Institute of Human Genetics:** J. Demaille, C. Ferraz, S. Eychenié et C. Lafont Shotgun library and sequencing
- **CIRAD:** P. Lagoda, X. Sabau Minipreps for BAC ends

Preparation of shotgun library

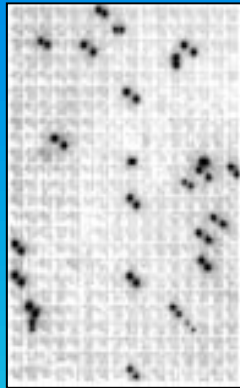
- Sonication of total DNA (1-2 kb fragments)
- Blunt-ended adapters added
- Ligated into pBluescript

Features of the *O. tauri* genome

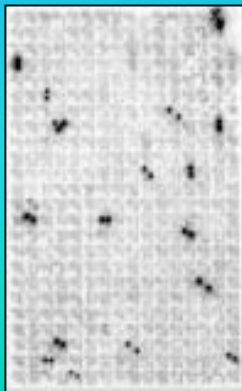
- ~50% genome sequenced- coverage ~1.2X
- 3627 contigs, ~3000 single sequences
- 5000-6000 genes of length ~2 kb
- Greatest homology with *A. thaliana* / higher plants
- Genes found with strong homology to:
 - cell cycle genes; cyclin A2, cyclin B1, cdk, map-kinase...
 - histones; H2A, H3 and H4
 - genes involved in transcription-translation, splicing machinery
 - helicases
 - ubiquitin pathway
 - photosynthesis genes
 - chloroplast and mitochondrion genes

Hybridisation of macroarrays and PFGE separated chromosomes

Find gene of interest
Idea of gene number

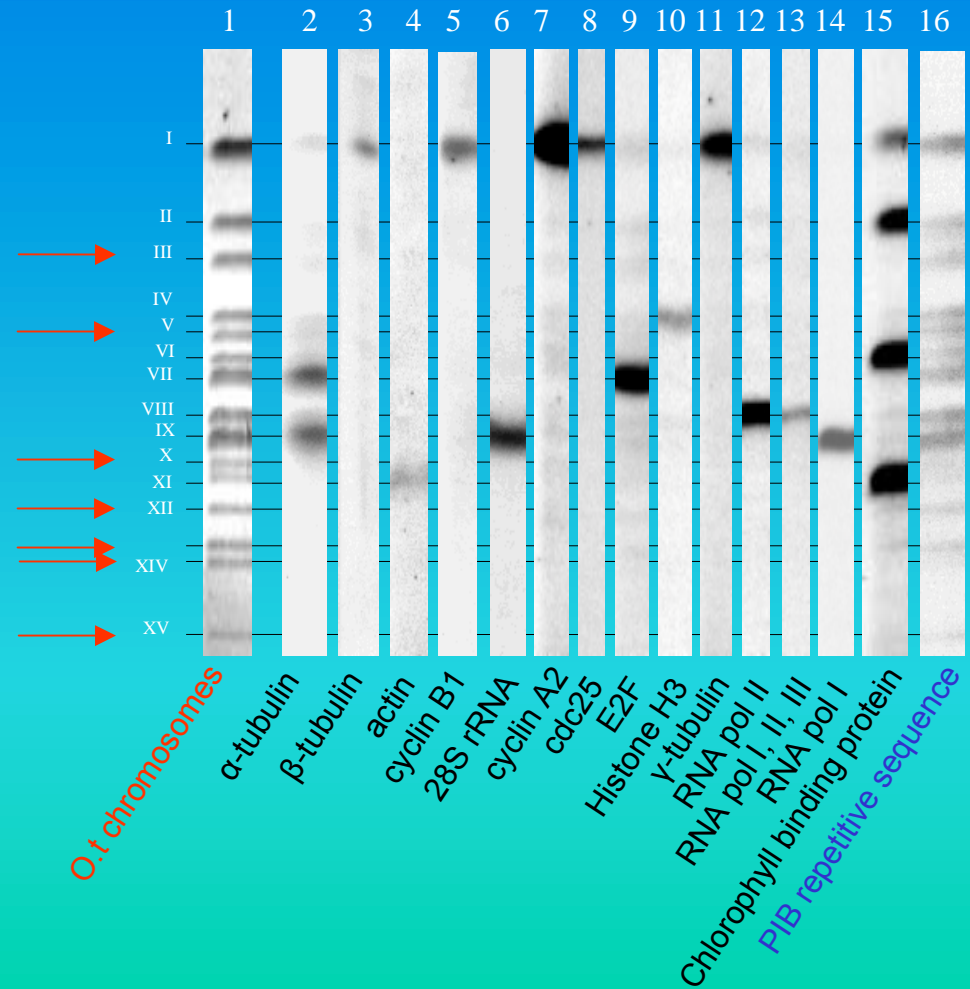


α -tubuline

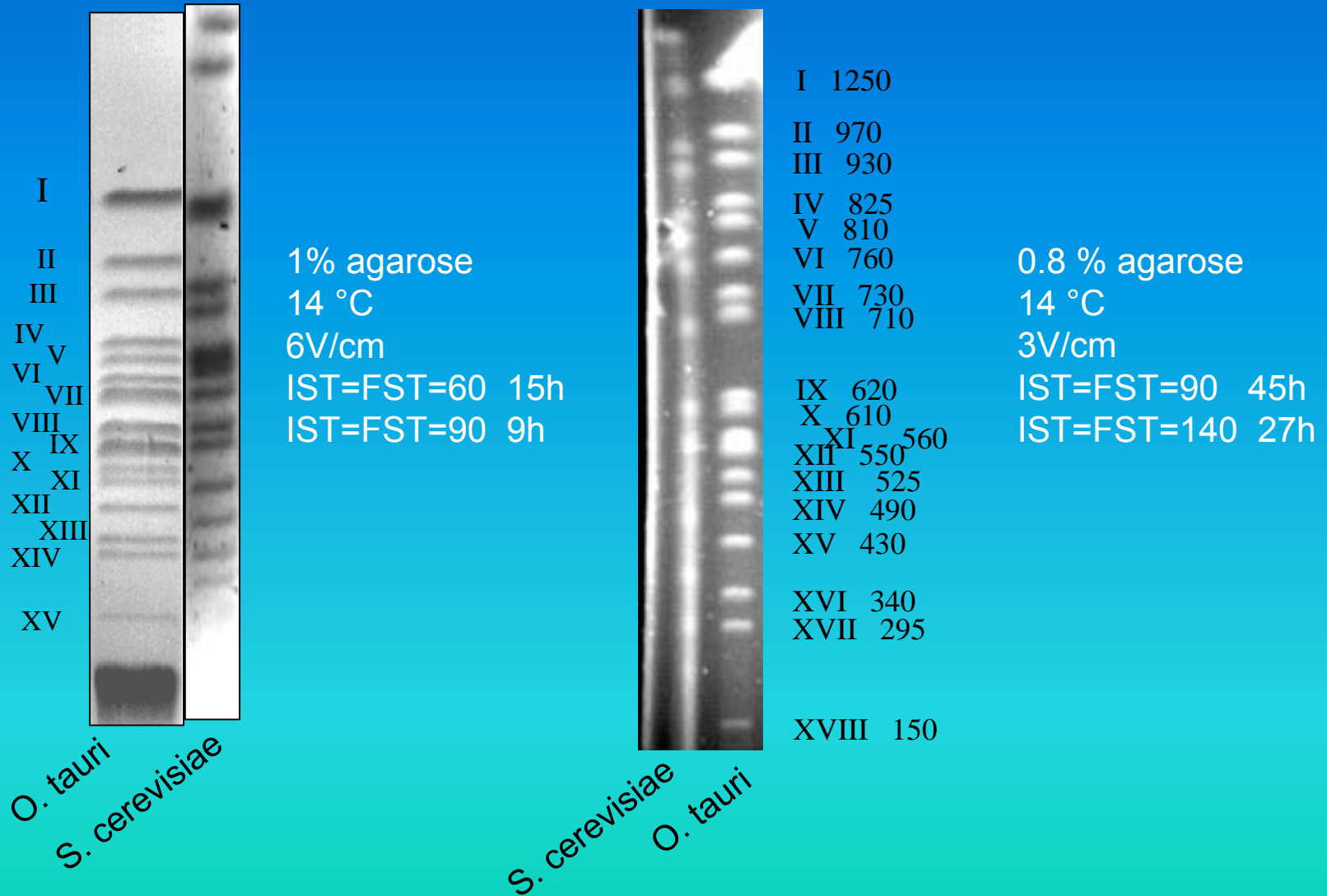


β -tubuline

Physical map of genes

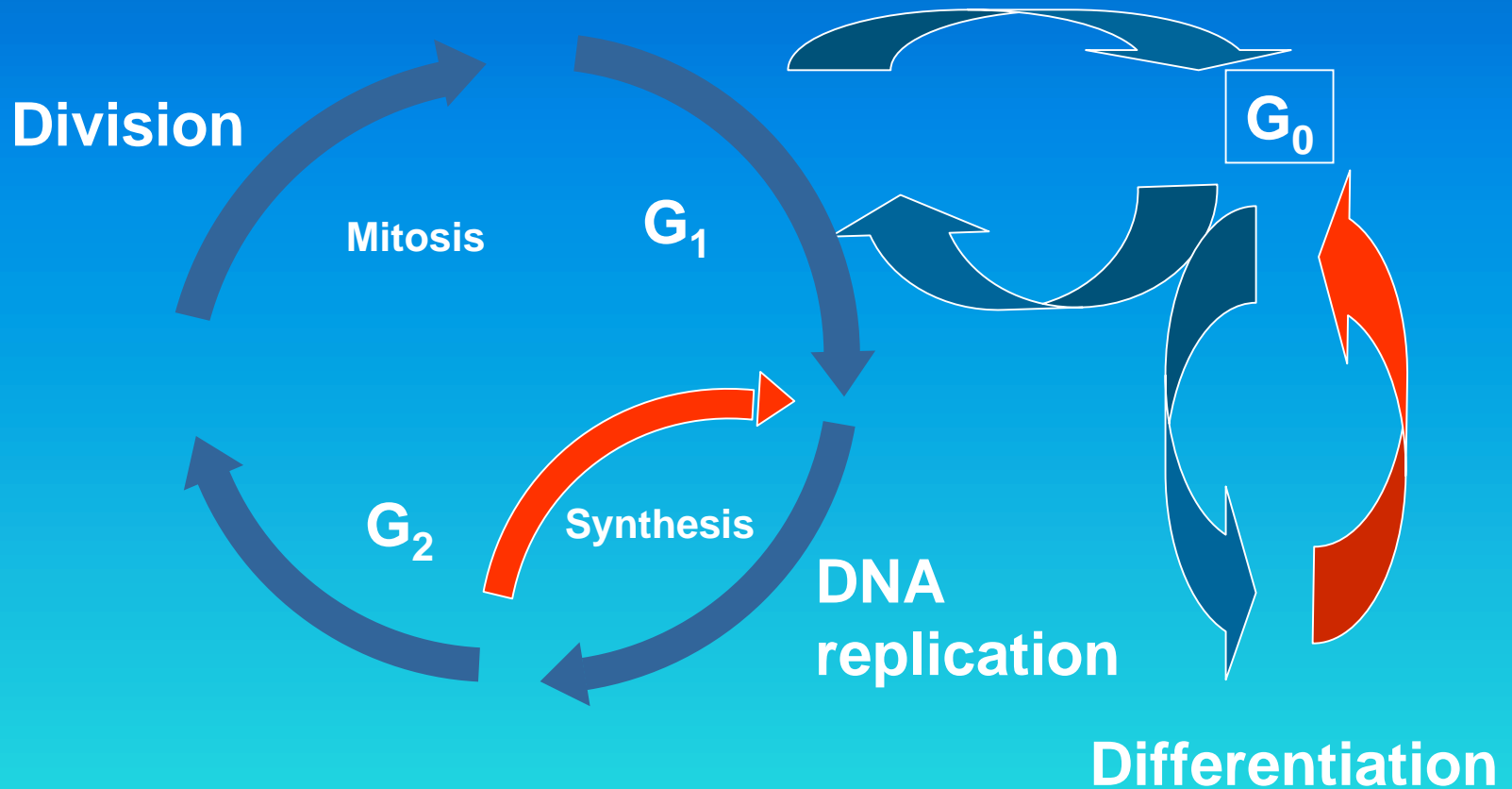


Recent PFGE of *O. tauri* chromosomes



✓15 chromosomes : genome of 9,71 Mb ✓18 chromosomes : genome of 11,56 Mb

The plant cell cycle

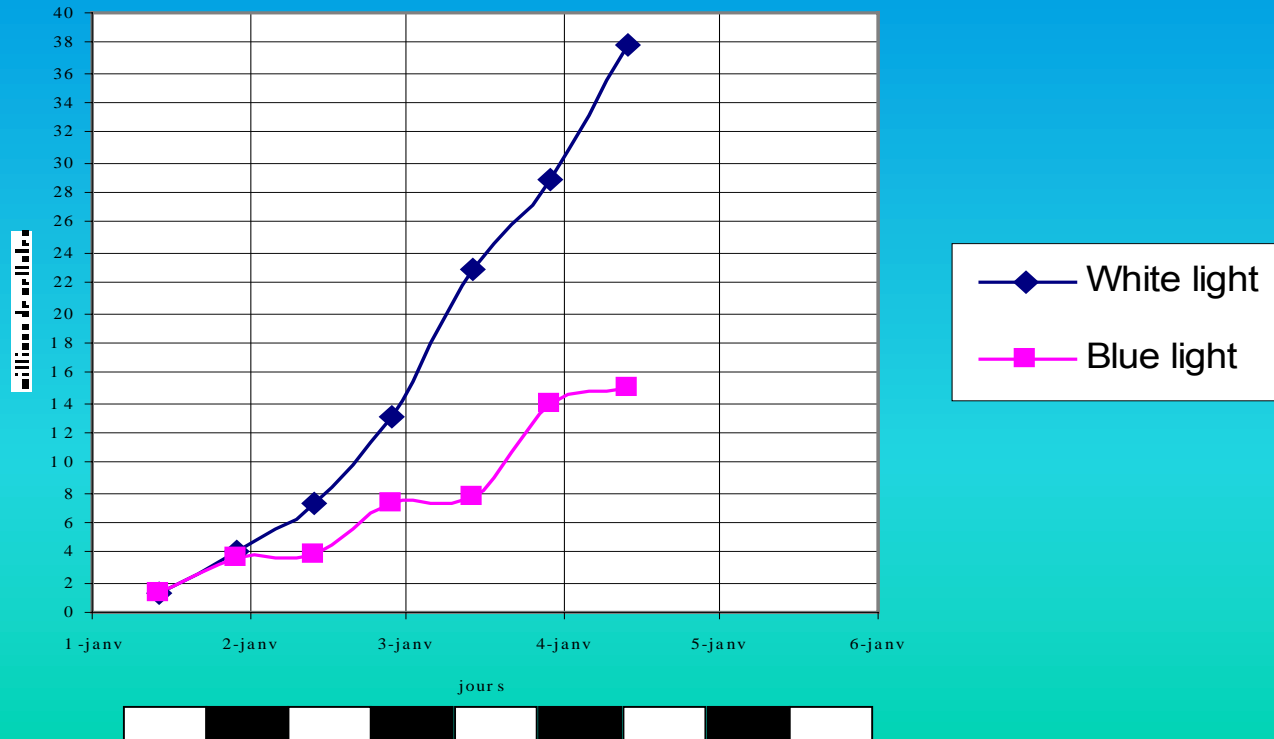


- Synchronisation important for cell cycle studies
- Provides a base for molecular and cell biology studies at each phase

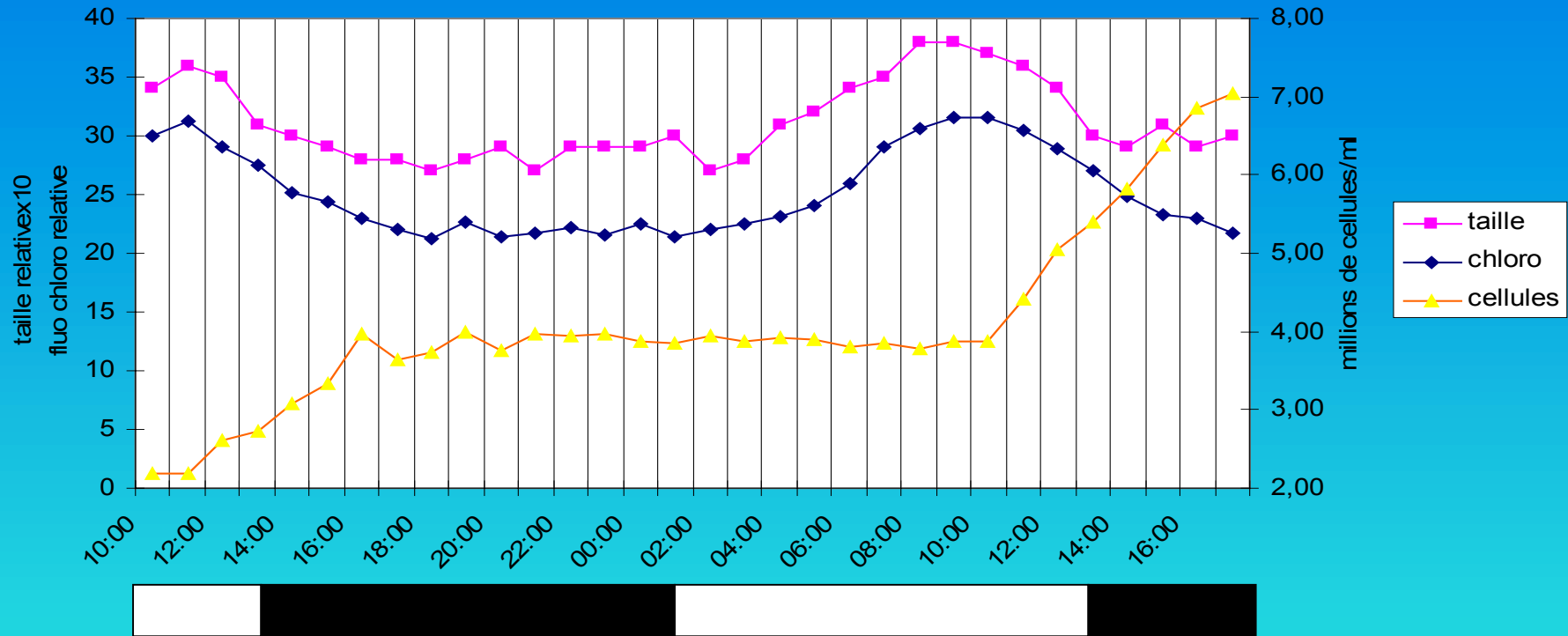
Effect of light period and intensity on growth

Conditions for 1 division/day

- 12h day/12h night
- 20 °C
- Blue light 3.2 μE

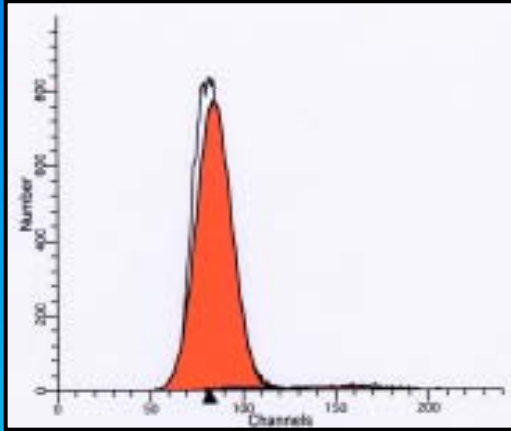


Characterisation of the 12/12 cycle

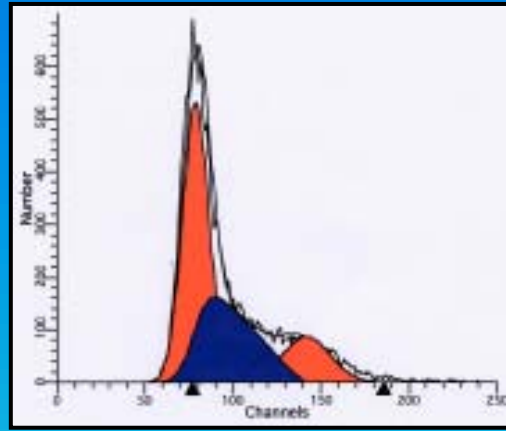


Variation of phases G1, S and G2-M during the 12/12 cycle

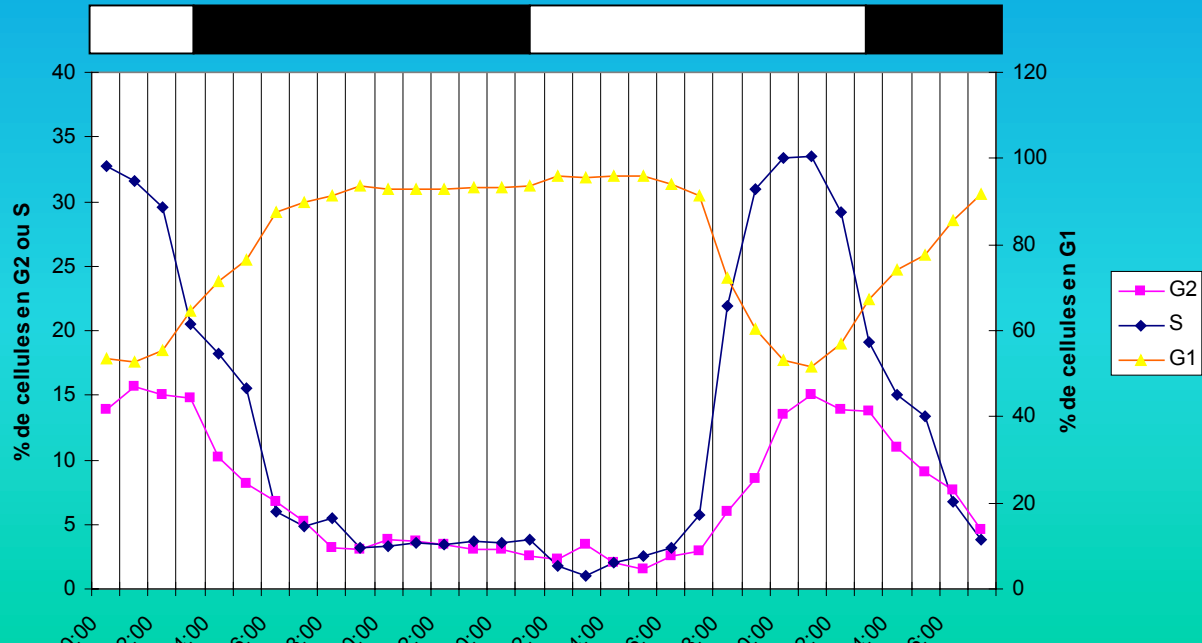
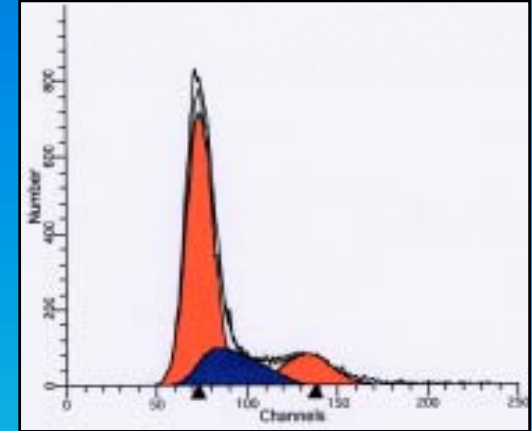
23h



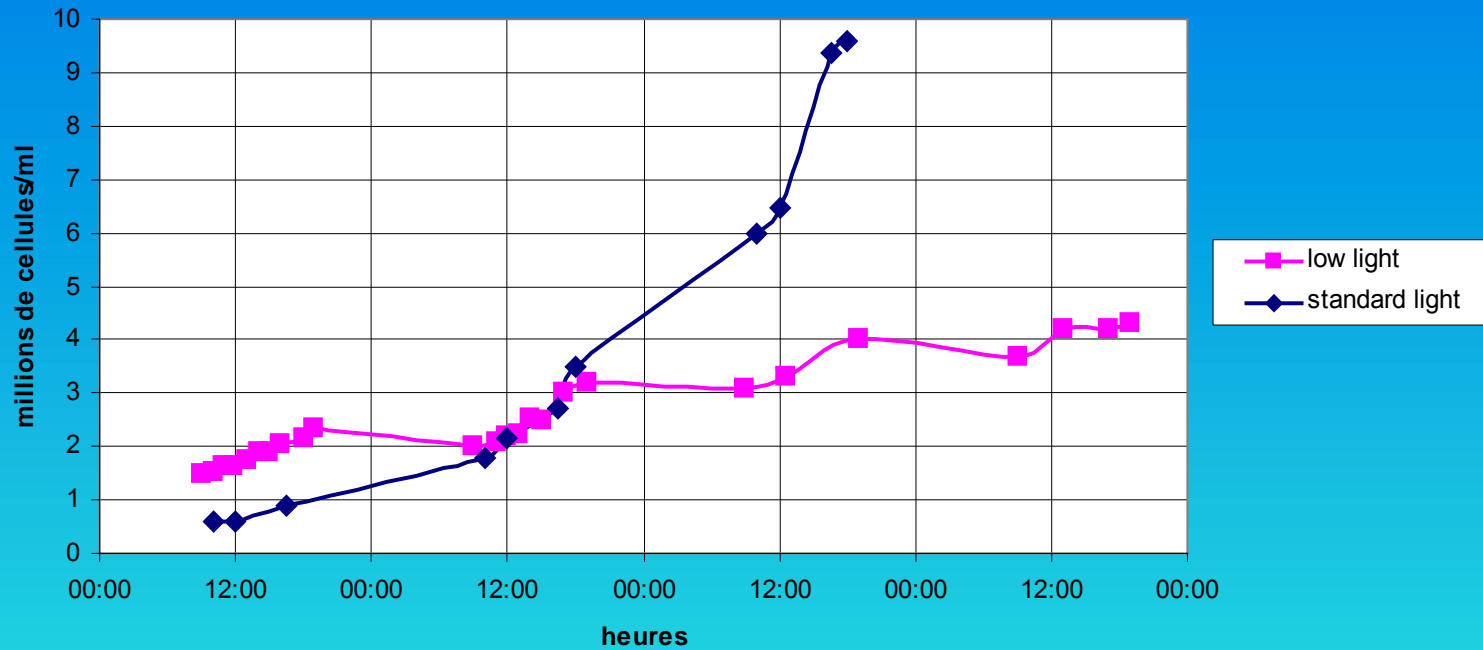
11h



13h



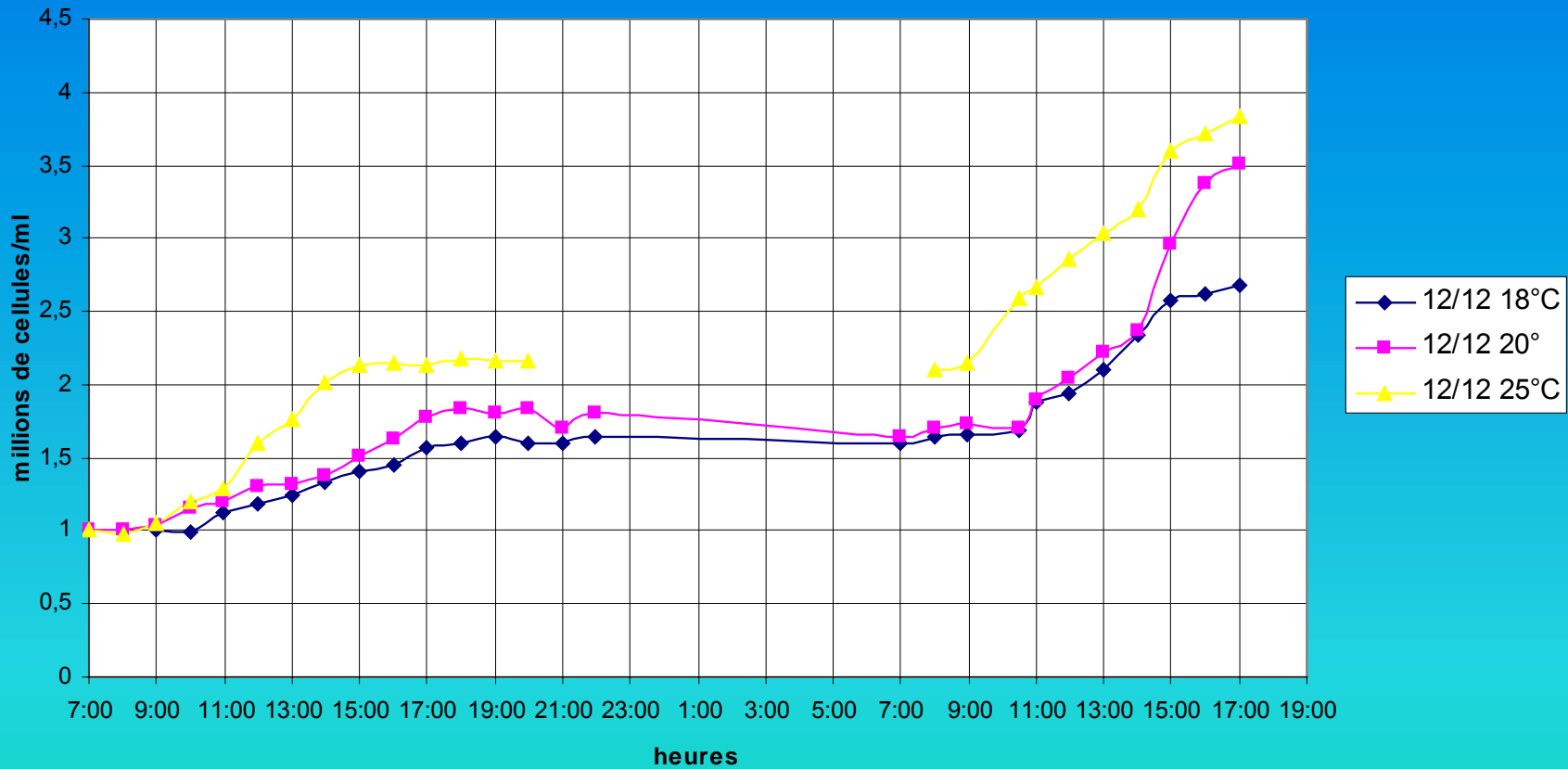
Growth of entrained culture under continuous blue light



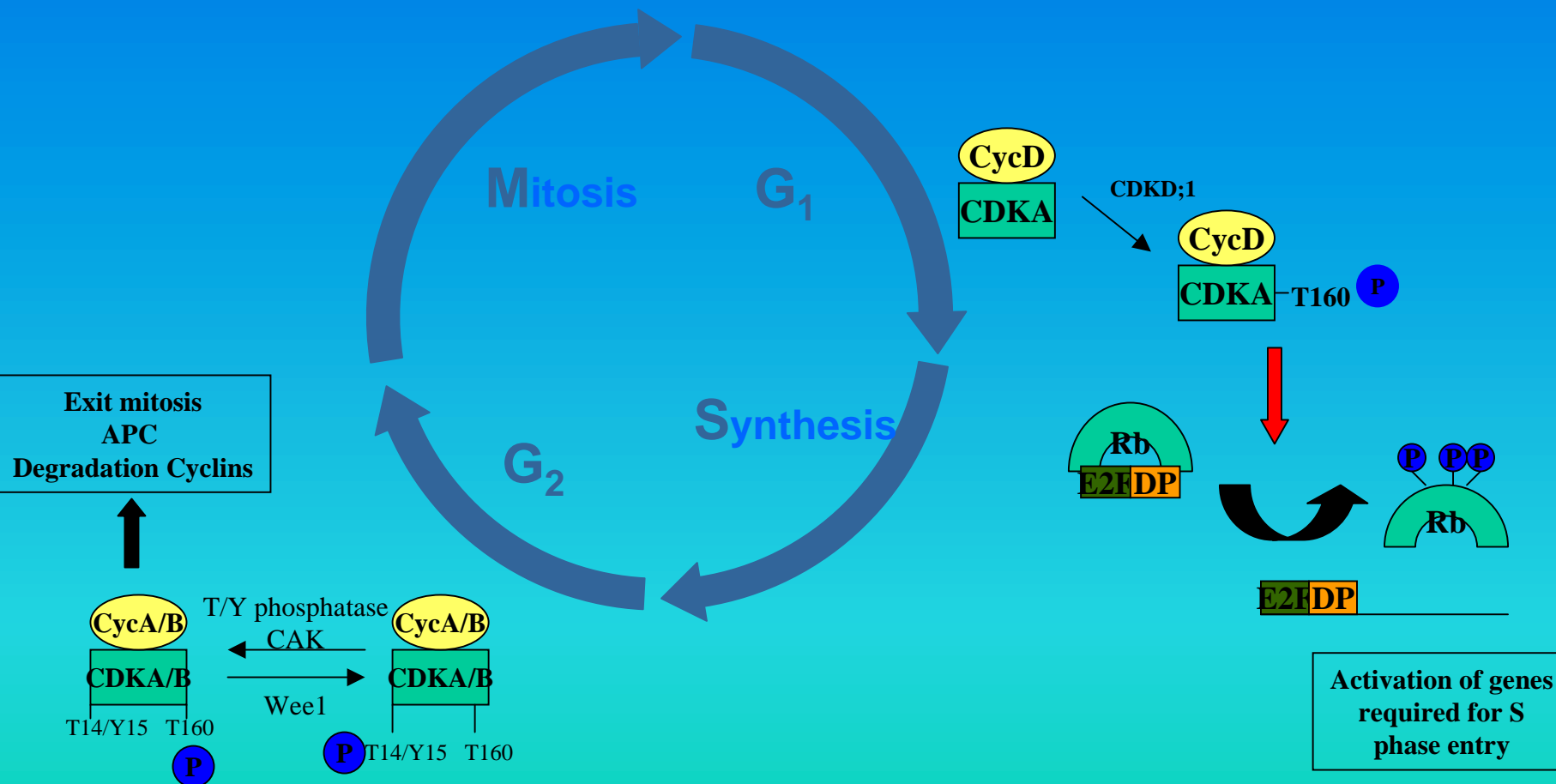
-low light intensity: $2\mu\text{E}$

-standard light intensity: $3.6\mu\text{E}$

Growth of entrained culture at different temperatures

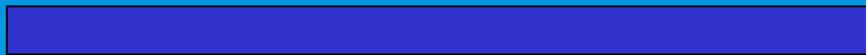


Regulation of the plant cell cycle



CDKs and cyclins found in *O.tauri*

PSTAIR



CDKA PSTAIR

PSTALRE



CDKB PPTALRE
PPTTLRE

CDKs



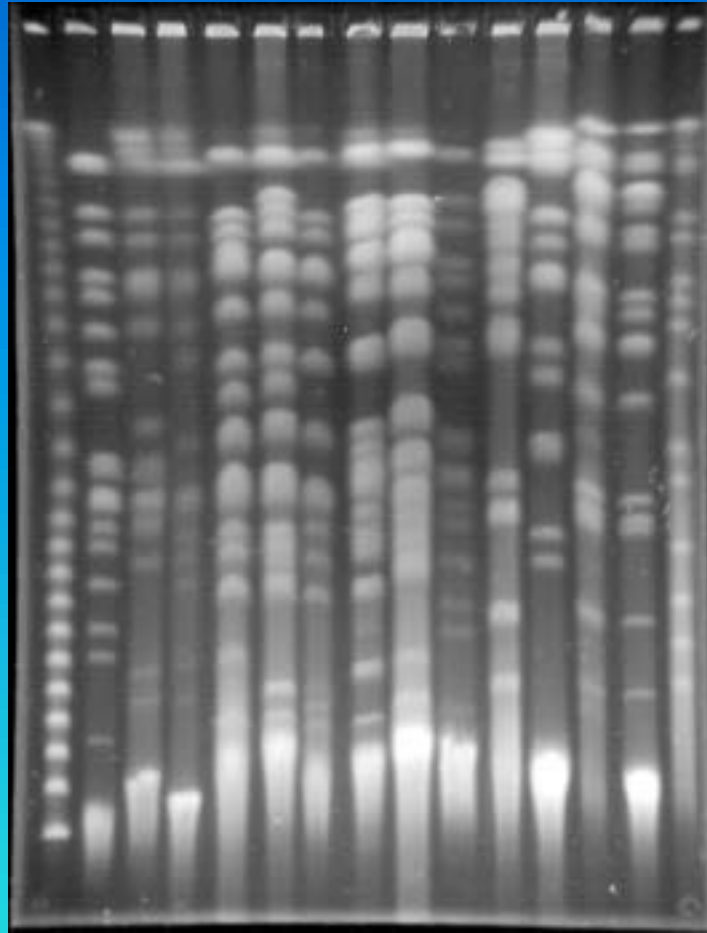
CycA



CycB1

Cyclines

PFGE typing of prasinophytes



Thau & Leucate lagoons
Tropical Atlantic ocean
Atlantic ocean, Morocco
Mediterranean sea / Sola
English channel

A. concatenate
O. tauri 1995, 0m
O. sp. 1991, 104m
O. oceanicus 1991, 120m
O. sp. 1999, 40m
O. sp. 1999, 65m
Ostreo. ? 1999, 0m
Ostreo. ? 2000, 0m
O. Tauri 2001, 0m
B. Prasinus, xm
Micromonas (BLA122)
B. sp. 2002, 0m
B. sp. 2002, 0m
S. cerevisiae

Future perspectives

- 1st annotated draft genome expected in 2003
- Annotation of mitochondrion and chloroplast genomes
- Physical map of genome
- Get complete sequences of CDKB-like, *cycA* and B-RACE PCR
- Characterisation of other potential cell cycle regulators
- Look for differential expression of genes and proteins
- Relationship between circadian clock and cell cycle
- Development of genetic manipulation system

The *O. tauri* team

Director CNRS UMR7628

–André Picard

Team leader

–Hervé Moreau

- **Genomics**
 - Evelyne Derelle
- **Genetic manipulation**
 - Nyree West
 - Evelyne Derelle
- **Study of natural populations**
 - Claude Courties
- **Cell biology**
 - Yvonne Bhaud
 - Rachid Bacchieri
 - Marie Albert
 - Dani Saint Hilaire
- **Cell cycle studies**
 - Florence Corellou
 - Basheer Khadaroo
 - Camille Mary