

*From Life Science to
Environmental Science:
Challenges and Successes of Molecular
Biology in the past 30 years*



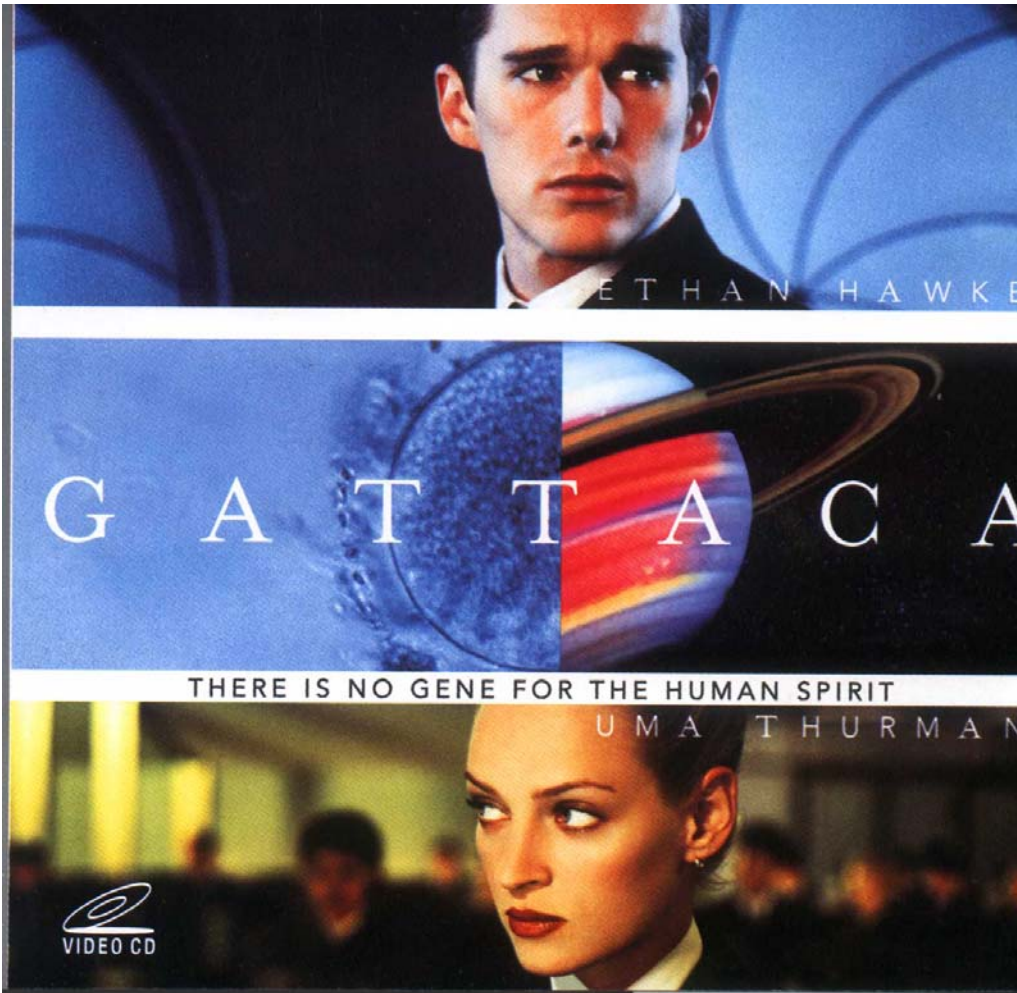
Teresa Lettieri

*European Commission Joint Research Centre;
Institute for Environment and Sustainability*

<http://ies.jrc.ec.europa.eu/>

<http://www.jrc.ec.europa.eu/>

The onset of Molecular Biology



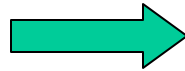
Insertion of piece of DNA
into a plasmid (vector) to
propagate into bacteria

How to “read” the DNA sequence
GATTACA

Insulin was the first therapeutic
recombinant protein

The onset of Molecular Biology

✓ **Cloning**



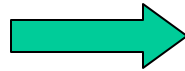
Insertion of piece of DNA
into a plasmid (vector) to
propagate into bacteria

✓ **Sequencing
technique**



How to “read” the DNA sequence
GATTACA

✓ **Expression of
recombinant protein**



Insulin was the first therapeutic
recombinant protein

First successes in Gene Cloning

Proc. Natl. Acad. Sci. USA
Vol. 76, No. 1, pp. 106–110, January 1979
Biochemistry

PNAS

Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

Expression in *Escherichia coli* of chemically synthesized genes for human insulin

(plasmid construction/*lac* operon/fused proteins/radioimmunoassay/peptide purification)

DAVID V. GOEDEL^{*†}, DENNIS G. KLEID^{*}, FRANCISCO BOLIVAR^{*}, HERBERT L. HEYNEKER^{*}, DANIEL G. YANSURA^{*}, ROBERTO CREA^{*‡}, TADAOKI HIROSE[‡], ADAM KRASZEWSKI[‡], KEIICHI ITAKURA[‡], AND ARTHUR D. RIGGS^{†‡}

^{*}Division of Molecular Biology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080; and [‡]Division of Biology, City of Hope National Medical Center, Duarte, California 91010

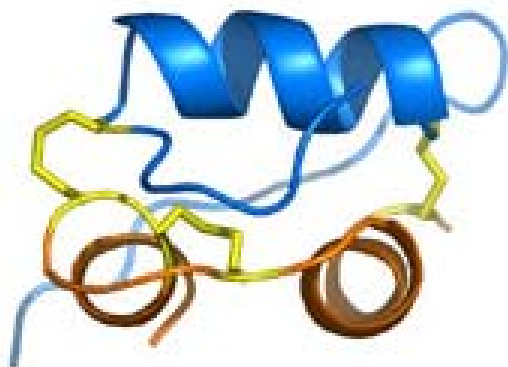
Nature Vol. 294 24/31 December 1981

cDNA sequences of human glucose 6-phosphate dehydrogenase cloned in pBR322

M. G. Persico, D. Toniolo, C. Nobile, M. D'Urso & L. Luzzatto^{*}

International Institute of Genetics and Biophysics, CNR, Napoli, Italy





Recombinant insulin

- Insulin was isolated in 1922
- For 60 years, cattle and pigs were the sources of insulin.
- In 1978, Genentech and City of Hope National Medical Center produced human insulin using recombinant DNA technology.
- ElyLilly licensed recombinant insulin from Genentech and developed it.
- In 1982, ElyLilly's Humulin became the first genetically engineered drug approved by the FDA.

From: C&EN SPECIAL ISSUE, The Top Pharmaceuticals That Changed The World, Vol. 83, Issue 25 (6/20/05)

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Glucose 6-phosphate dehydrogenase (G6PD) enzyme

G6PD' s role in red blood cells is to provide molecules enable to protect against oxidative stress generated by several oxidant agents

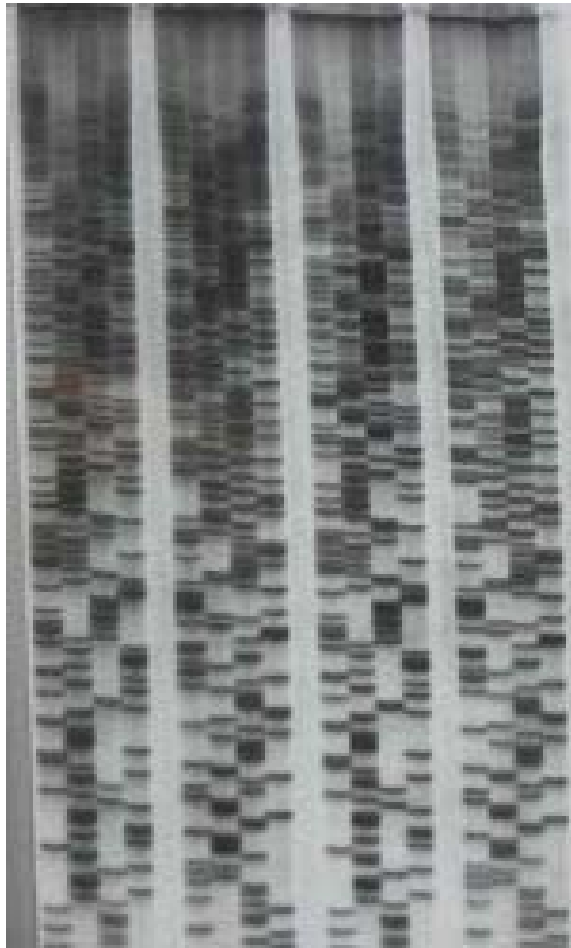
- G6PD deficiency is the most common human enzyme defect, present in more than 400 million people worldwide

→ Hemolysis induced by drug (e.g. drug for malaria)

→ Favism: Hemolytic anemia induced by ingesting fava beans

→ Hereditary non spherocytic hemolytic anemia

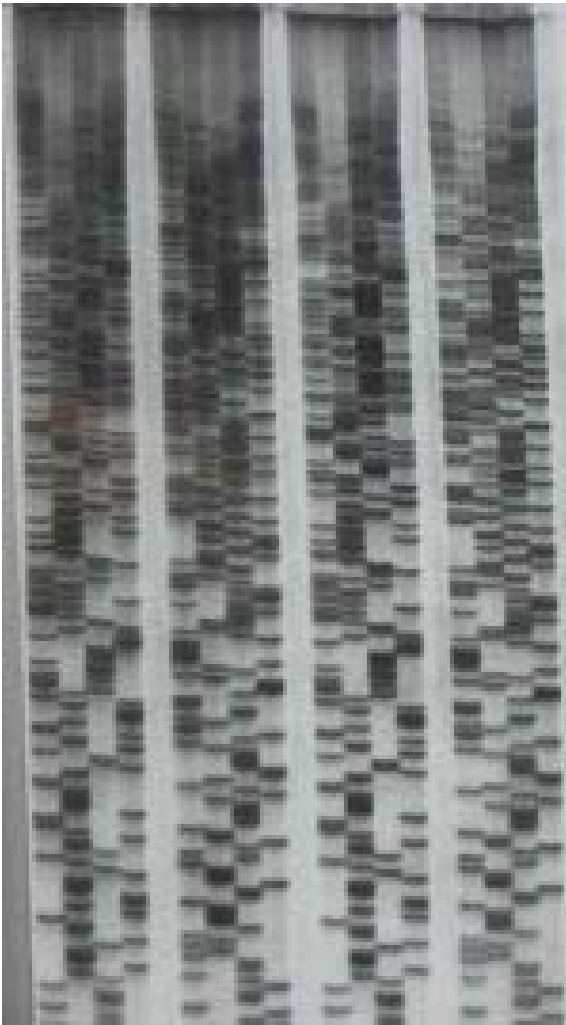
Sequencing method



CATG CATG

Big challenge at that time:
sequencing G6PD from several
individuals to identify the mutations

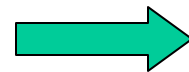
The automation of sequencing



CATG CATG

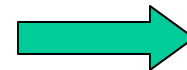


1 capillary

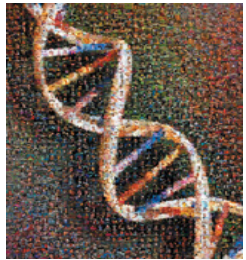


96 samples

96 capillaries

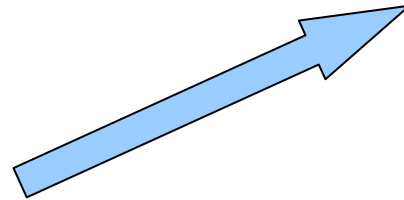


96 samples
at the same time!!



Nature, 15 February 2001

Genome Sequencing

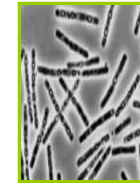
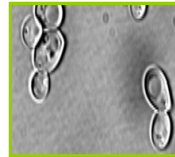
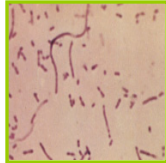


Haemophilus influenzae
The first organism to be
sequenced

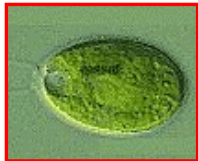


Completed in 2003

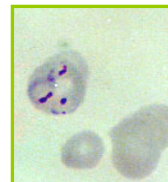
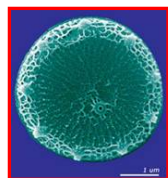
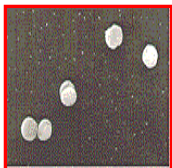
Some considerations



✓ *At beginning the sequencing was concentrated on pathogens or species relevant for biomedical field*

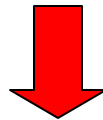


✓ Many other organisms relevant in environment field were sequenced

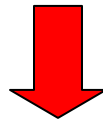


DNA Microarray Technology

✓ *High density spatial synthesis on chip*

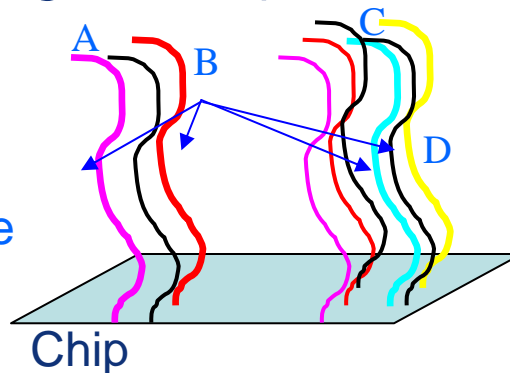


✓ *Thousands of genes or the whole genome on the chip*



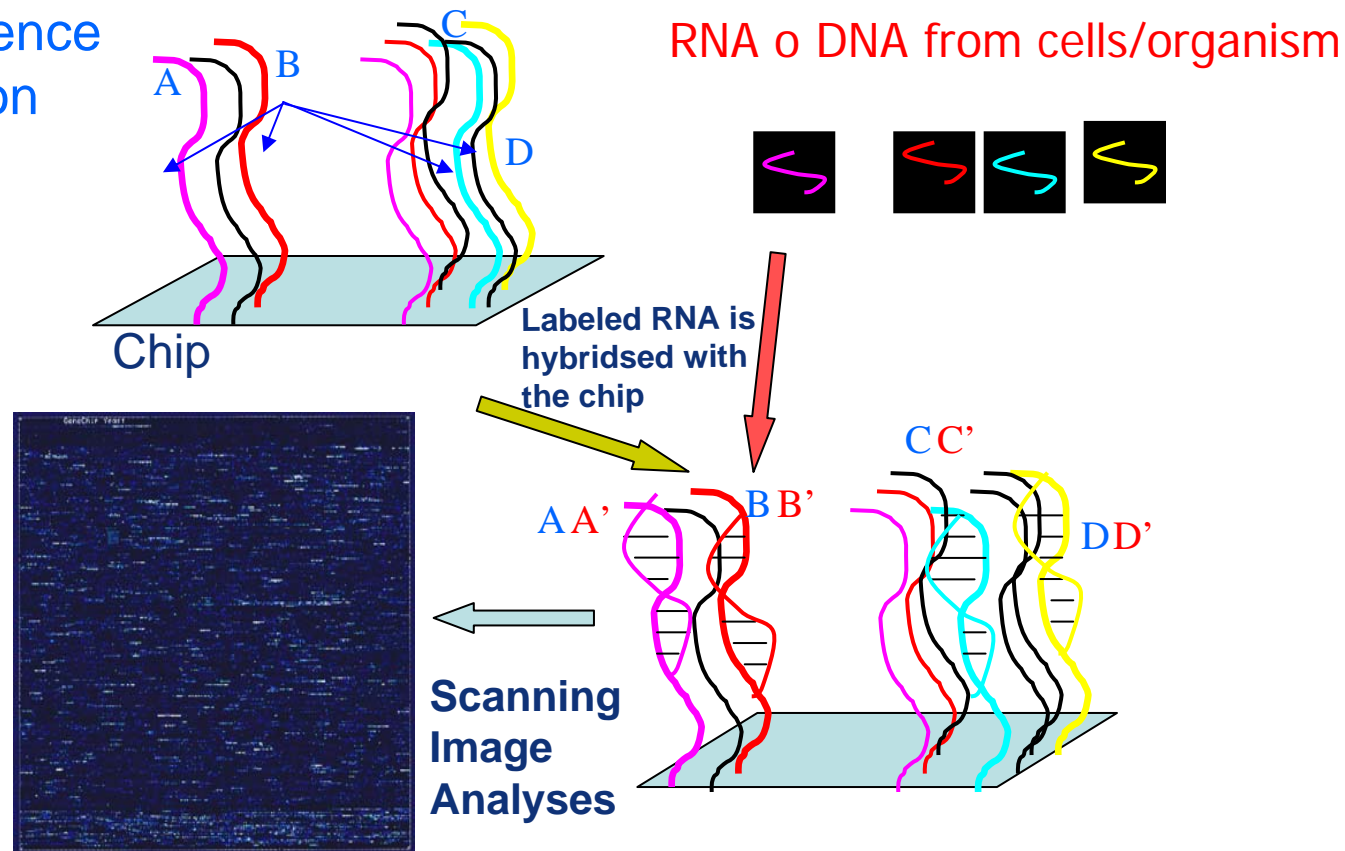
✓ *Possibility to analyze the expression level of several genes (or the whole genome) at the same time*

Part of each
gene sequence
is spotted on
the chip



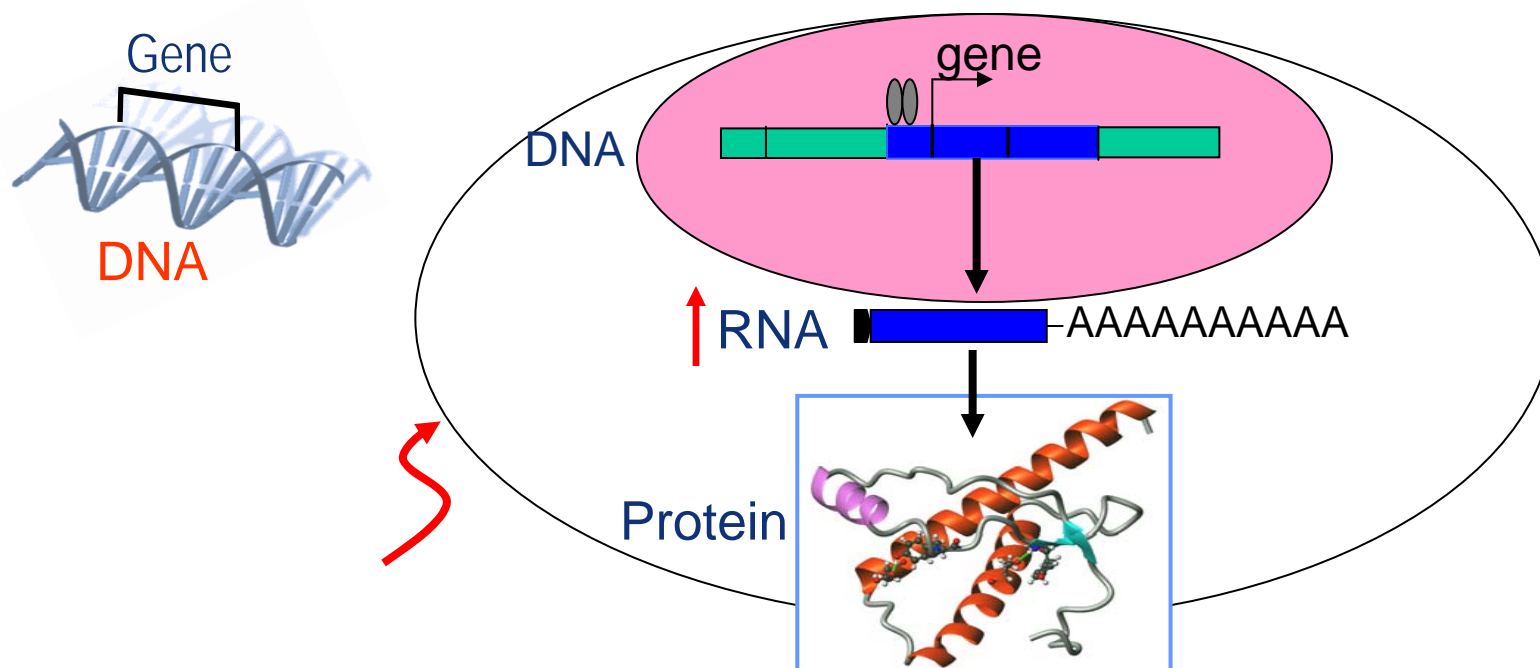
DNA Microarray

Part of each
gene sequence
is spotted on
the chip



The fluorescence signal is directly proportional to the abundance of RNA

Inside the cell/ organisms

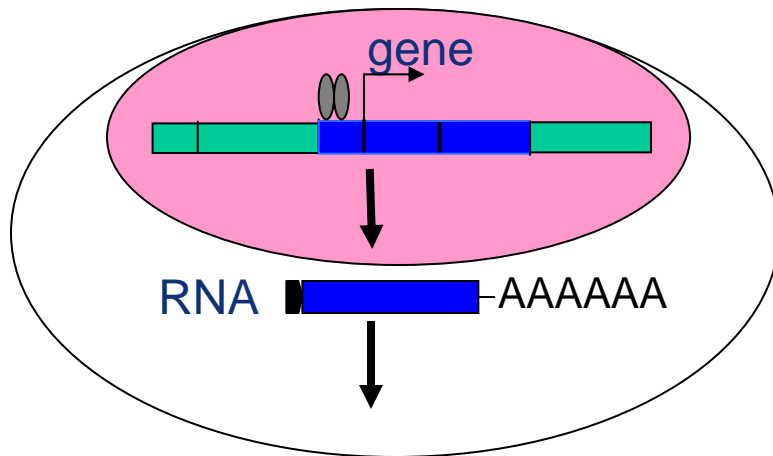


Stressors can induce changes at expression level such an increase ↑ or decrease ↓ of RNA)

(drug, chemical pollutants, UV lights)

Gene Expression Profile

Normal



Tumor

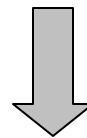
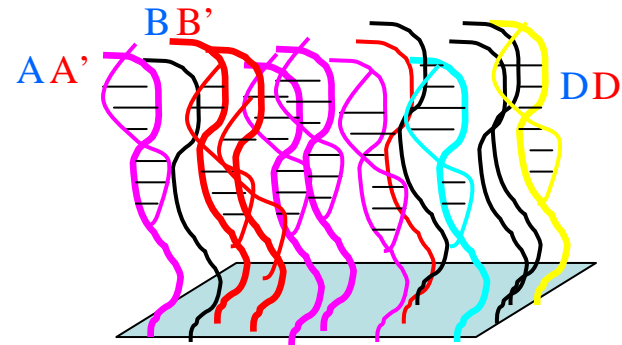
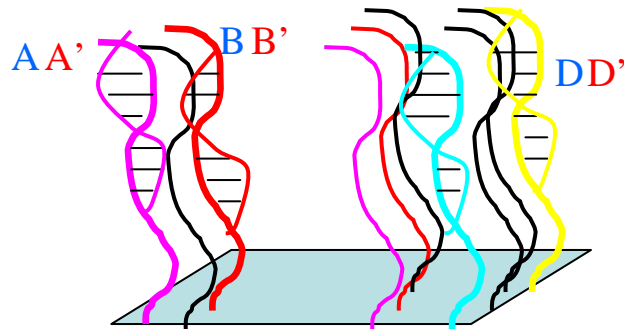
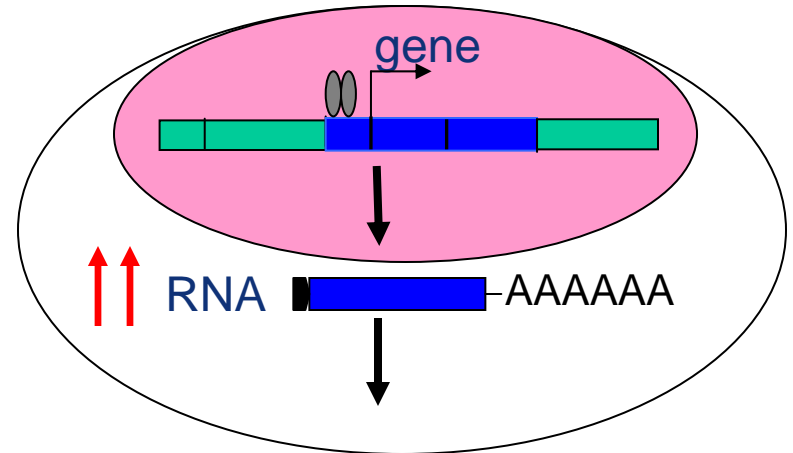
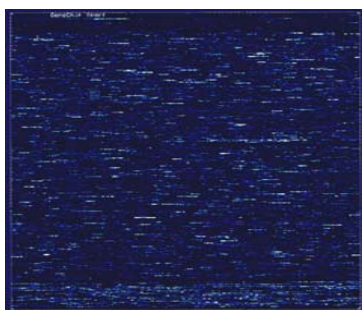
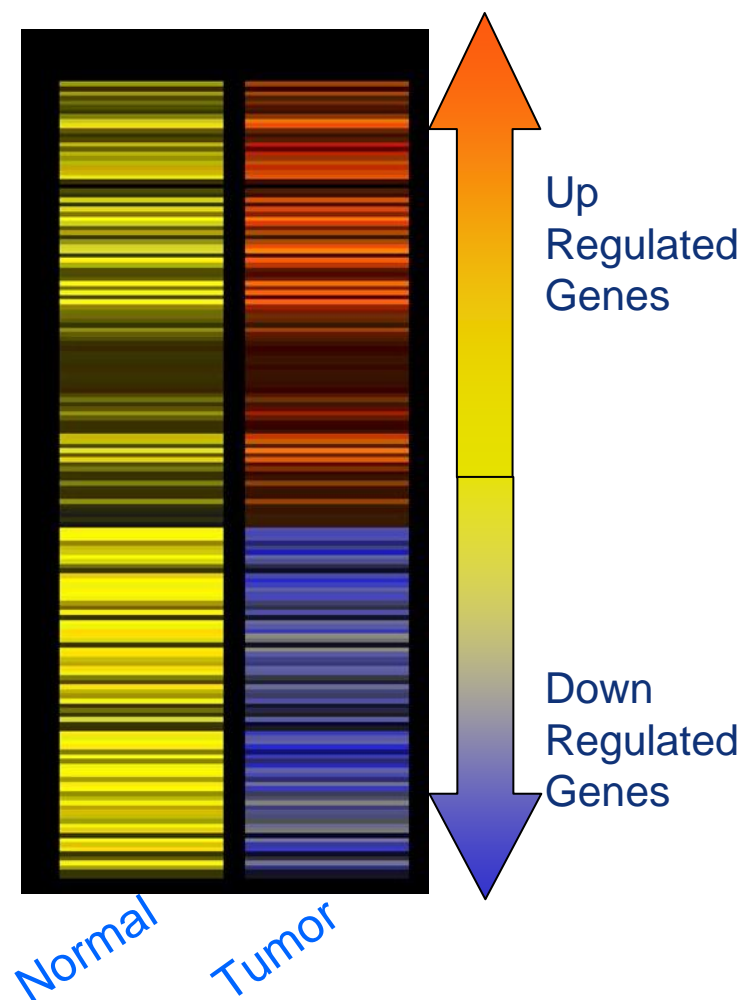


Image scanning of the chip

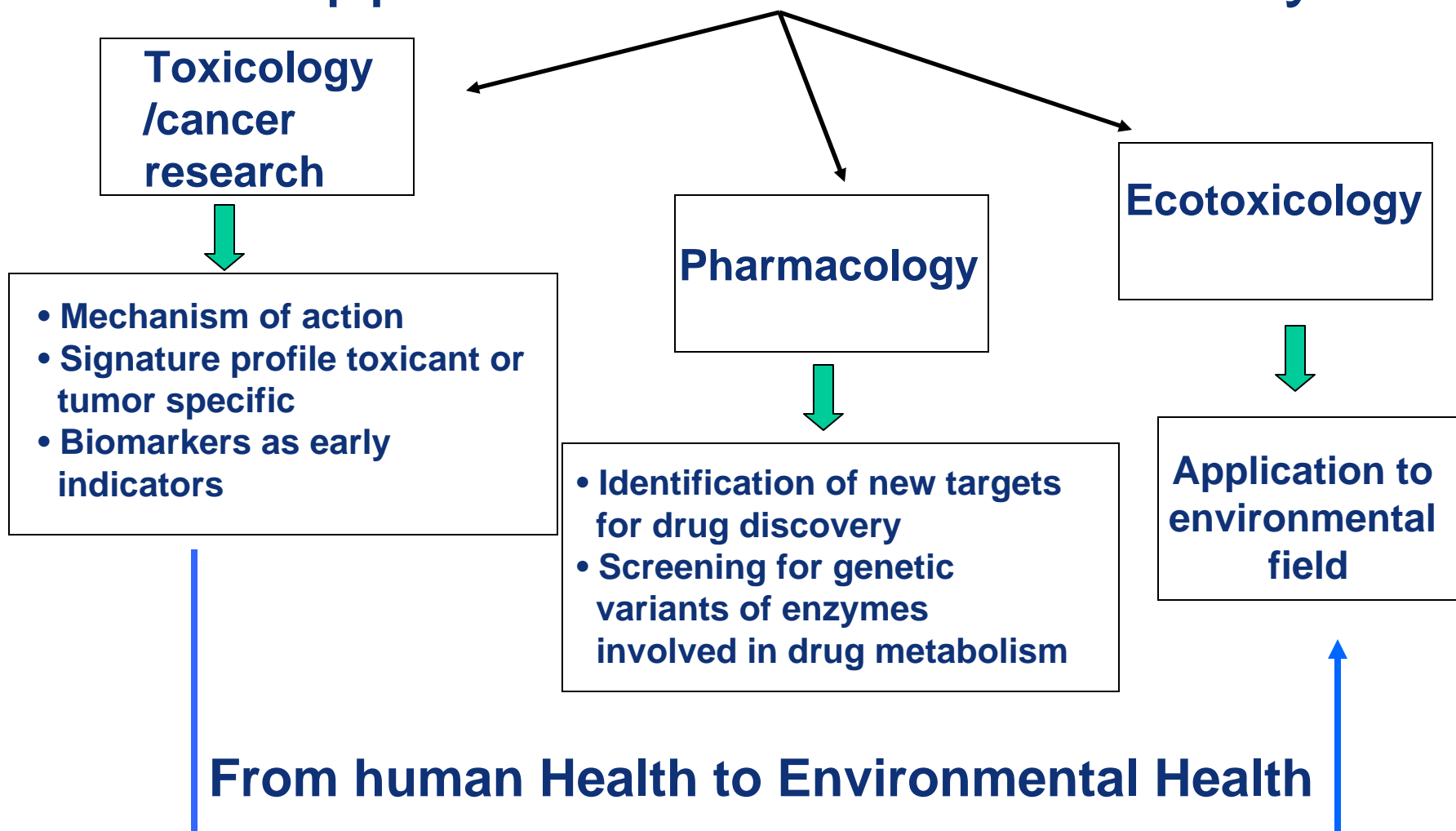
Gene Expression Profile



- ✓ Gene profile of tumor cell compared to normal cells → Markers
- ✓ Identify new targets for drug development



Application of DNA Microarray

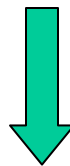


DNA Microarray to Environmental sciences

Why?

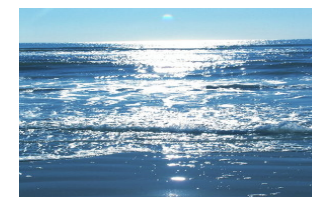


Exposed to anthropogenic pollutants
such as pesticides, polyhydrocarbons,
pharmaceutical compounds



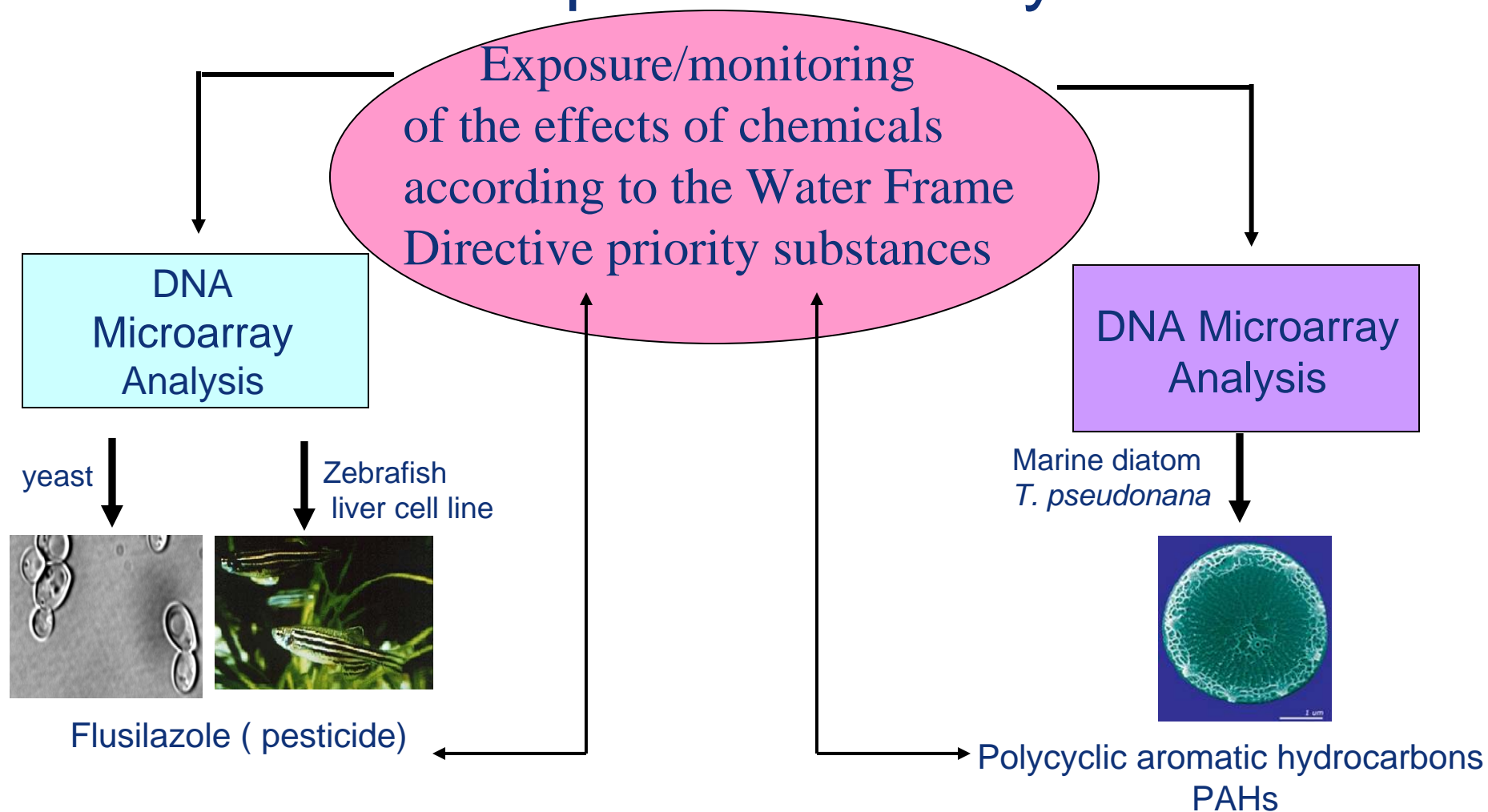
All components of the ecosystems
(fishes, mammals, algae, bacteria, viruses)

DNA Microarray allows:

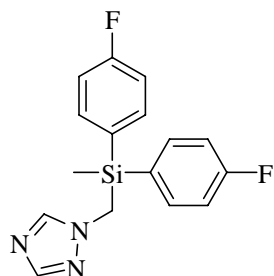


- Understanding the mechanism of toxicity of the pollutant on the organisms
- Identify a gene expression profile which could be specific for pollutant
- Identification of some of genes as early indicators of the exposure to toxicants so to prevent long-term exposure and subsequent deleterious effects

Gene expression analysis

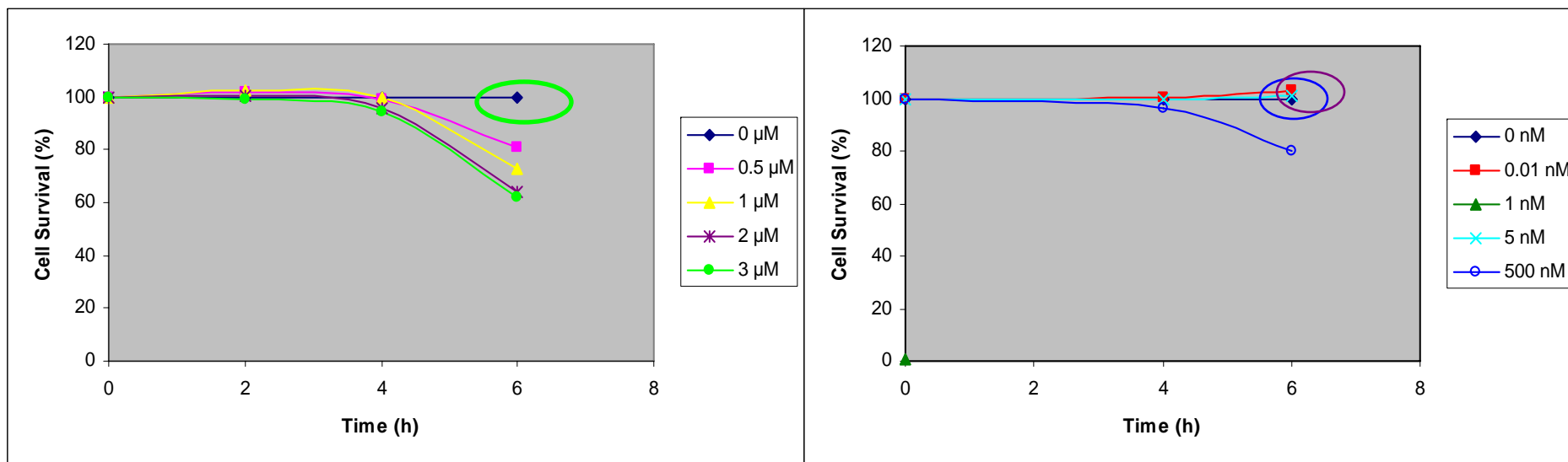


Flusilazole



Flusilazole

- Fungicide widely used in agriculture in Europe and in other countries
- Belongs to the triazole chemical family
- It is a sterol synthesis inhibitor, which interferes with fungal membrane structure and function

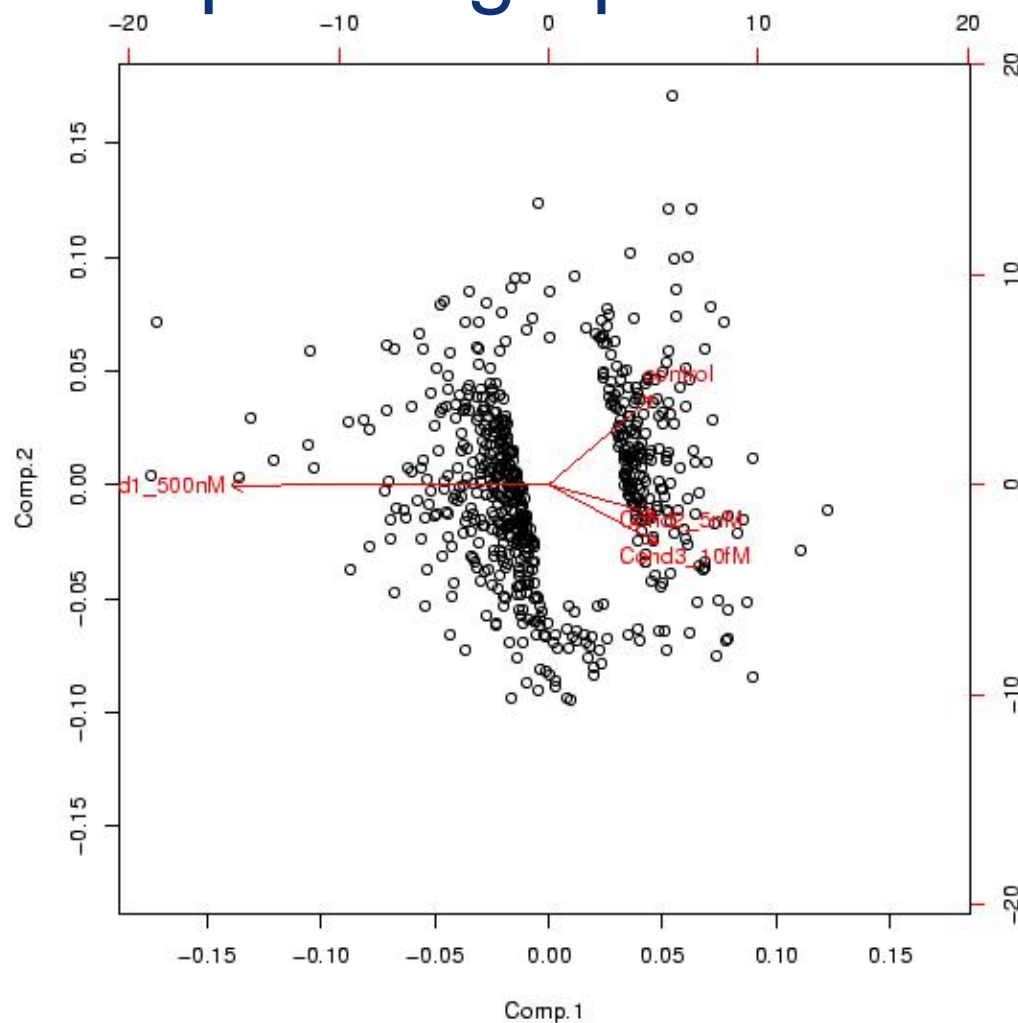


 500 nM → 20% mortality

 5 nM → No effect (NOEC)

 0.01 nM → Environmental concentration (< 3.3 µg/L)

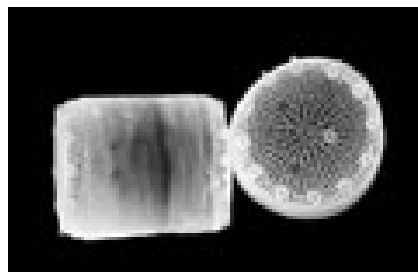
Principal Component Analysis of Gene expression profiling upon flusilazole exposure



- control
- cond1_500 nM
- cond2_5nM
- cond3_10 fM

Cytotoxic effects

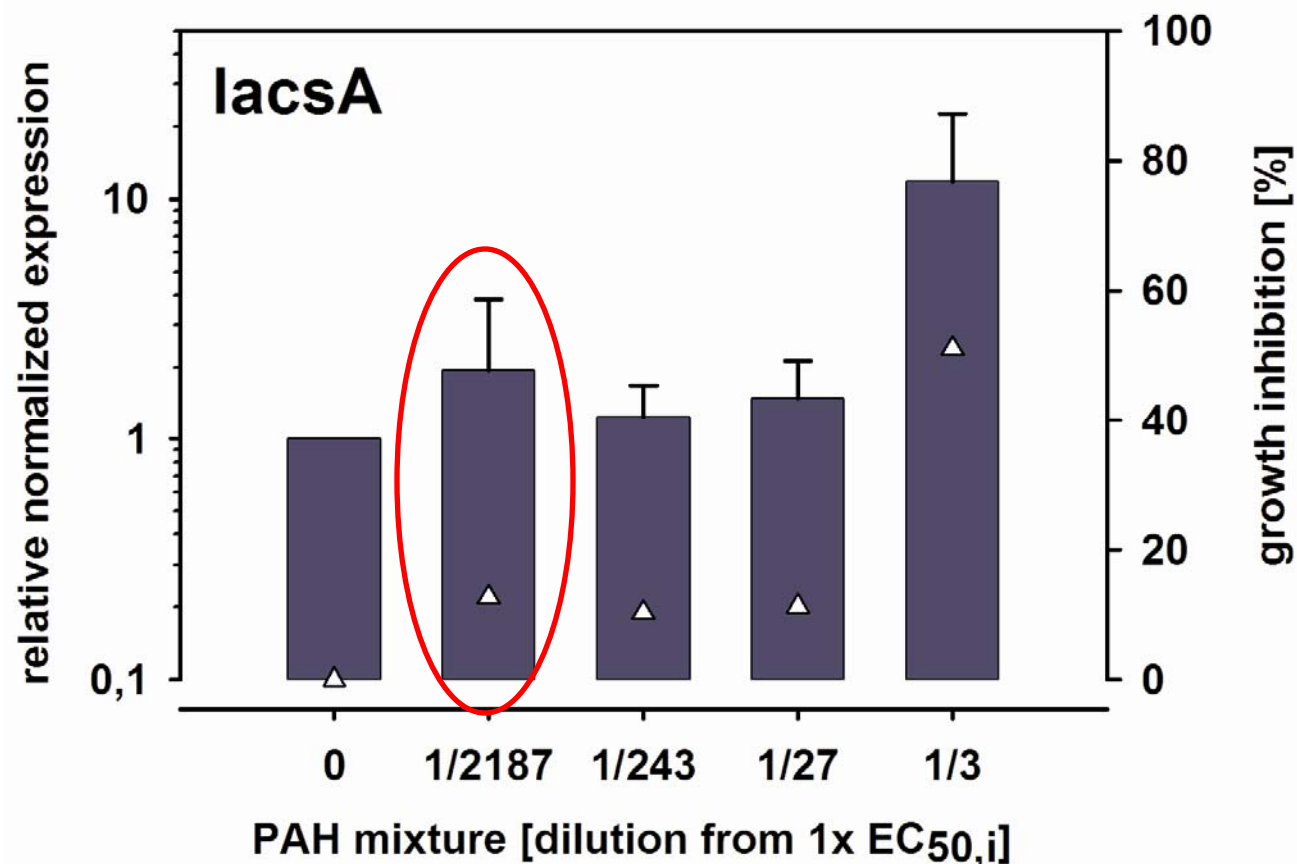
Dose-response curves
→ Effect concentrations



Effects on gene expression at mRNA level

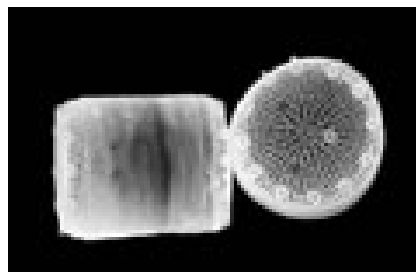
Fatty acid metabolism

lacsA: Long chain acyl-coA synthetase is involved in fatty acid metabolism



Cytotoxic effects

Dose-response curves
→ Effect concentrations



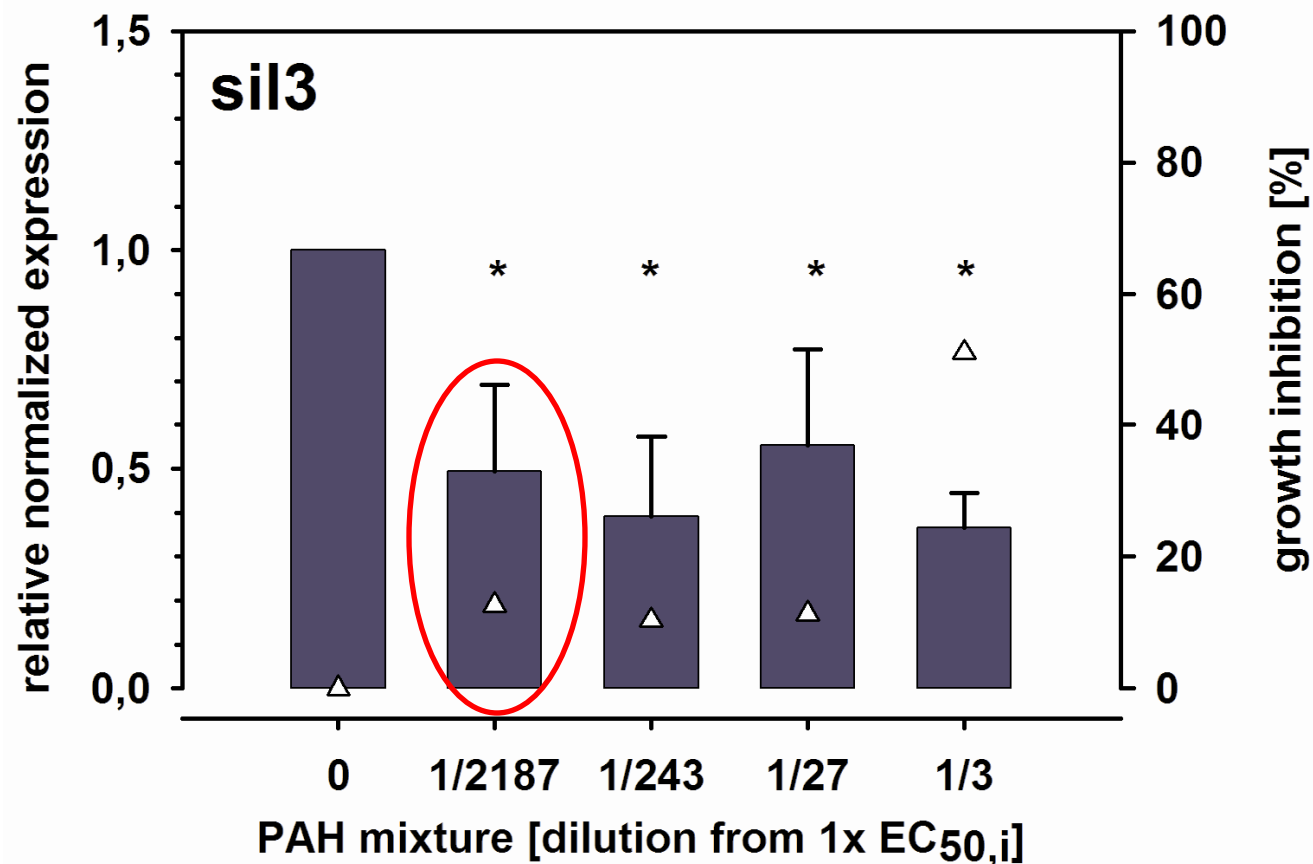
Effects on gene expression at mRNA level

Silica shell formation

Sil3: silaffin precursor 3 is involved in silica shell formation



Silaffin transporter is down regulated

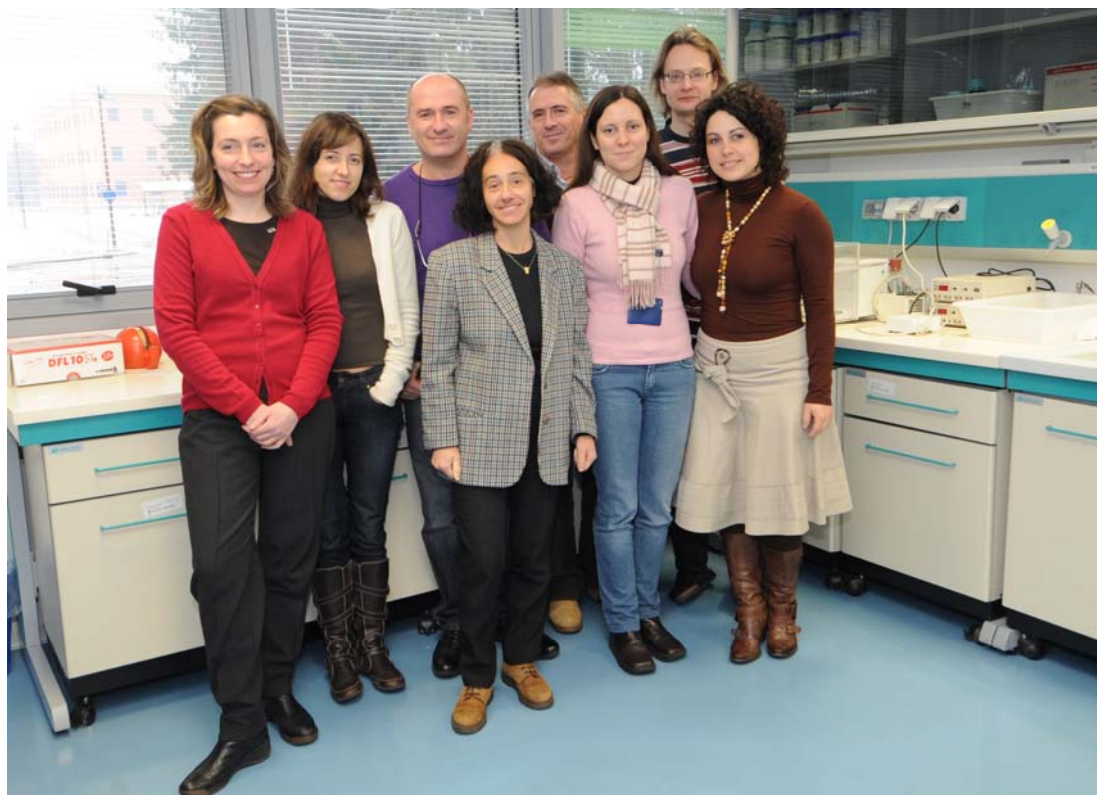


Conclusion / Remarks

- ❖ Molecular Biology has been extremely successful in helping us to understand how genomic information is translated into biology
- ❖ It has provided tools to improve health, for example specific and early diagnostics and biological drugs (e.g. Insulin)
- ❖ The applications to environmental sciences is at an earlier stage due to the more complex problems and the many variables in a real ecosystem
- ❖ A better defined regulatory environment and increased international collaborations could play an important role in addressing urgent problems in environmental sciences

The Future will be....

Molecular Environmental Biology



Thank you for your attention