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MARIE CURIE ACTIONS

"Gene expression profile of biomarkers: an early warning sentinel of environmental pollutants? "

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The BIOECOTOX project (EIF-2003-501356), Discipline: Environment & Geoscience

Ecotoxicology and the Molecular Biology Approach

The BIOECOTOX project is expected to serve the "Global change and Ecosystem" priorities of the 6th EU framework program for research (FP6). One of its major research topics is to develop a better understanding of ecosystem functioning to minimise the negative impact of human activities and to ensure the sustainable management of natural resources.

Recently biomarkers, defined as indicators of physiological alteration in organisms, have emerged as important tools in environmental risk assessment. However, the ecological factors and the absence of specificity to pollutants may complicate interpretation of the significance of their responses. Molecular biomarkers in ecotoxicology offer the possibility of providing (1) early detection of environmental stress, (2) inferred mechanisms of action and (3) efficient monitoring of the environment. The promise is that the knowledge of toxicogenomic responses in one species may help predict the modes-of-action of similar agents in other species and risk assessment in humans.

The BIOECOTOX project focuses on the gene expression profile of selected biomarkers using a very sensitive technique: the RT-PCR. This method should improve the sensitivity and the specificity of the pollutants detection. For this purpose, model organisms are exposed to selected priority hazardous substances.

The RT-PCR

The RT-PCR (Real Time-Polymerase Chain Reaction) is the most sensitive technique for quantifying changes in gene expression (Fig.1). During the process, the mRNA is cyclically amplified and the detection of PCR products on a real-time basis allows to measure very small changes of gene expression.

Probes and primers are designed to hybridize the target gene of interest. The probes have a fluorescent dye (R) and a quencher (Q) that prevents the detection of fluorescent signal. During the extension phase of the PCR, the cleavage of the probe separates the fluorescent dye from quencher, increasing the fluorescent signal. (Fig.2). Thus, the increasing fluorescence in each cycle, is proportional to the amount of probe cleavage (Fig.3).

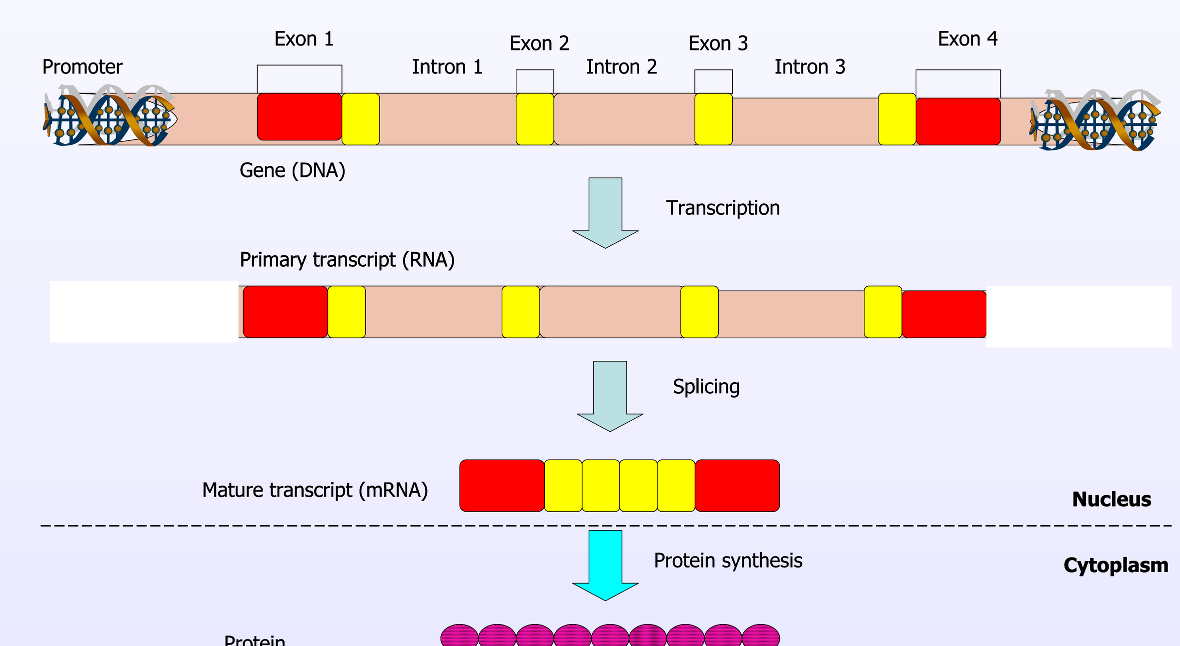

Figure 1. Simplified overview of gene structure and expression. A protein-coding gene is first transcribed to yield a primary transcript, which is processed to remove the introns. The mature transcript (mRNA) is then translated into a sequence of amino acids, which defines the protein.

Preliminary results

Acute toxicity of Benzo(a)pyrene in the yeast *Saccaromyces cerevisiae*

We have tested 5 concentrations of B(a)P (0 to 1500 ng.l^{-1}) on *S. cerevisiae* at 5 exposure times (10mn to 48h). For comparative quantification, we determined a reference gene called house keeping gene (HK) that is not affected by the chemical treatment.

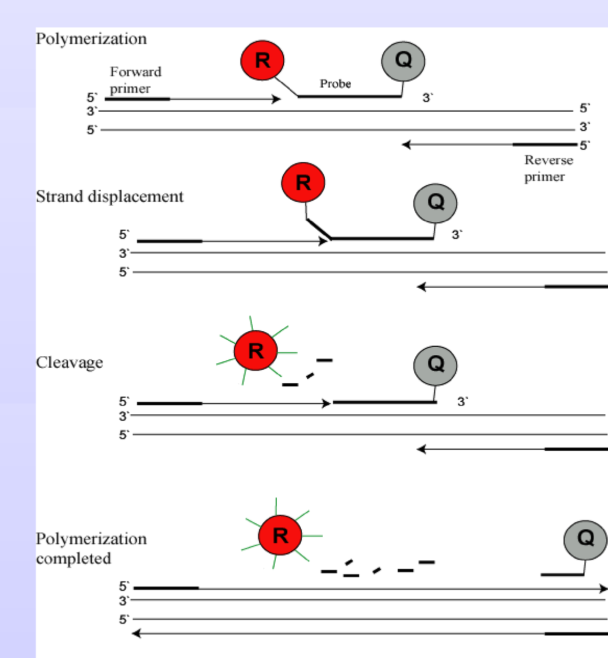

Figure 2. Representation of one cycle of amplification in RT-PCR

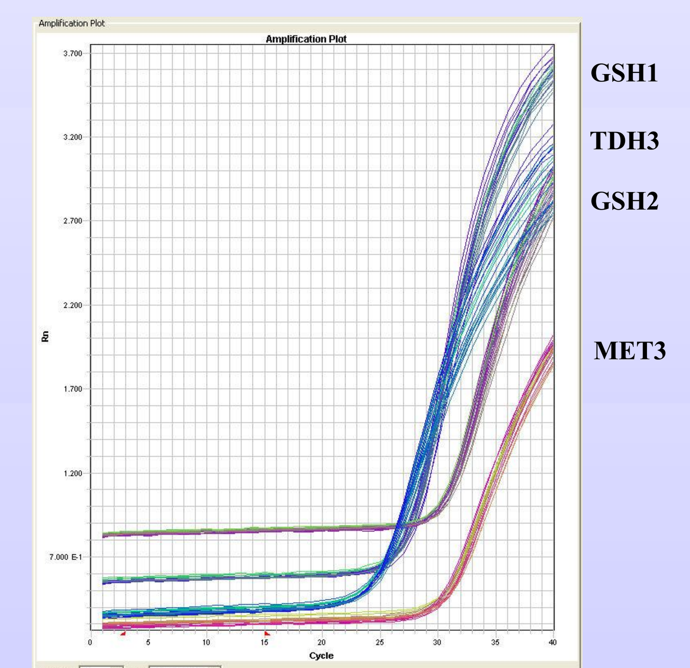

Figure 3. RT-PCR amplification plots of four different genes. 3 targets (GSH1, GSH2, MET3) and 1 HK (TDH3)

House keeping test:

TDH3, ACT1 and 18S genes were analyzed by RT-PCR at three of the concentrations and three of the exposure times tested. The TDH3 gene exhibited the highest Pearson correlation factors related to the two other genes and thus were chosen as the reference gene.

Biomarkers test:

Genes involved in the phase I (ERG3, ERG5, ERG11) and phase II (GSH1, GSH2, MET3) detoxification pathway of B(a)P have been tested. Preliminary statistical analyses (Two way ANOVA) show no significant difference among the concentrations. In contrast a significant difference occurs in the exposure time responses.

Two "profiles" are observed (One way ANOVA): see Figure 4

(1) Down expression compared to the early stage level (10mn), for all the target gene except ERG5, at 30mn and/or 2h exposure and later at 24h and/or 48h

(2) Up expression, compared to the early stage level (10mn), after a medium and a long exposure for ERG5 (2h/ 48h) and GSH2 (4h/24h and 48h)

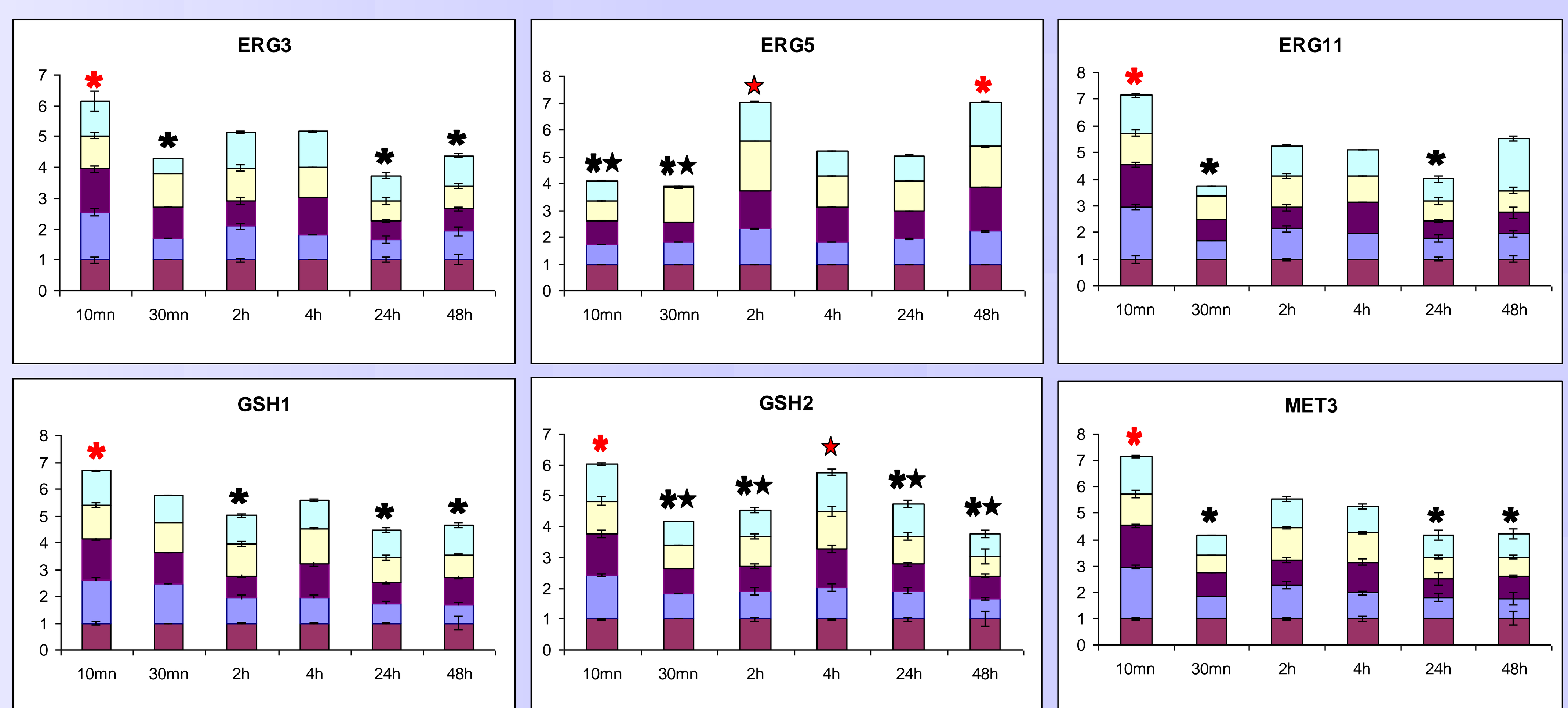

Figure 4. Expression level of target genes in S. cerevisiae exposed to B(a)P. The results are expressed relatively at the HK gene and the non treated sample (0 ng.l^{-1}). The figure represent for each gene the sum of the expression levels measured at the different concentrations tested and according to the exposure time. 0 ng.l^{-1} 10 ng.l^{-1} 50 ng.l^{-1} 250 ng.l^{-1} 1500 ng.l^{-1}

One way ANOVA analysis: * significantly different ($p< 0.05$) of * ; ★ significantly different ($p< 0.05$) of ★

Conclusion

Preliminary results show that the gene expression level of selected genes is more linked to the exposure time rather than to the B(a)P concentration. The profile of the gene expression is complex and probably involves different metabolic regulations. Other biomarkers should be tested to understand the complete detoxification mechanism. Further experiments on different organisms and chemicals (single or mixture) will be performed to have a more complete set of data to validate this method for a sensitive and specific environmental pollutants detection.

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