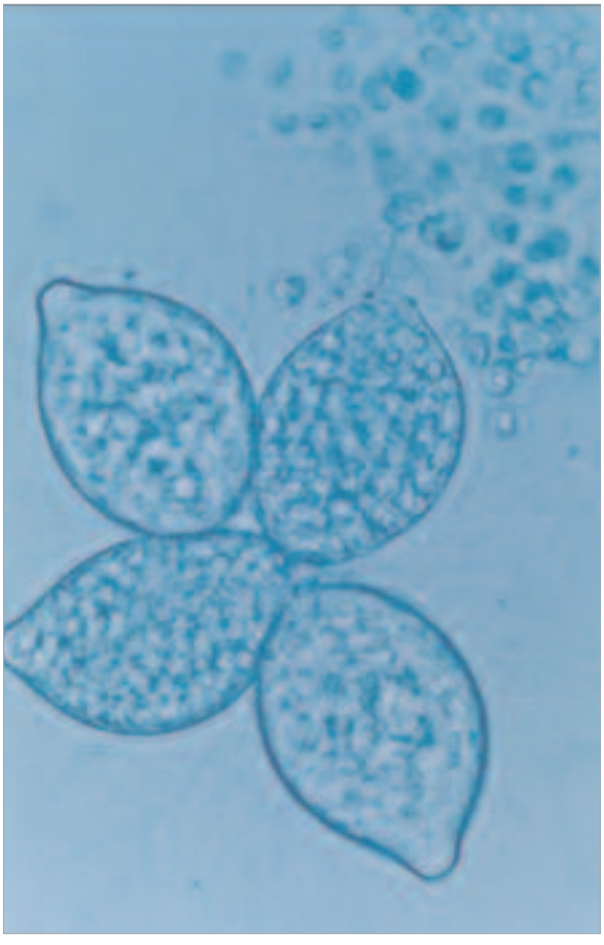
GENIC EXPRESSION REGULATION IN MICROORGANISMS

Suely Lopes GOMES

Chemistry Institute / University of São Paulo



Sporulation of B. emersonii

This research project aims at investigating the molecular aspects related to the control of gene expression during cell differentiation, heat shock and other stress conditions utilizing as model systems: the gram-negative bacterium *Caulobacter crescentus* and the chytridiomycete *Blastocladiella emersonii*. In *Caulobacter crescentus*, we intend to investigate a putative homologue of the gene *hspR* (CC0081), which in other bacteria encodes a repressor that controls the expression of several heat shock genes. The HSPR protein represses gene expression by binding to an inverted repeat present in the regulatory region of the genes, denominated HAIR (HspR associated inverted repeat). For these studies we will construct a null mutant in gen Cc0081, whose phenotype will be analyzed with respect to different kinds of environmental stresses. Another objective of this project deals with the characterization of 5 ECF (extracytoplasmic function) sigma factors from *Caulobacter*: *SigF, SigU, SigT, SigE* and *SigR*. Concerning *SigF*, which we recently showed to be involved in the response to hydrogen peroxide in stationary phase cells, we will investigate the molecular mechanisms controlling its activity. Preliminary results indicate that *SigF* is post-transcriptionally regulated during stationary phase. In addition, gene Cc3252, which forms with *SigF* a probable operon, will be investigated as a possible anti-sigma factor. *SigU* and *SigT*, whose expression is induced by osmotic stress, will be better characterized, looking for *Caulobacter* genes that are regulated by these sigma factors. Null mutants of *SigE* and *SigR* will be obtained and their phenotypes analyzed, to uncover the role of these sigma factors in the control of gene expression in this bacterium. We also propose to characterize expressed sequence tags (ESTs) from *Blastocladiella emersonii* exposed to heat shock or Cadmium. In the case of exposure to Cadmium, very little is known with respect to the primary targets of this heavy metal. With the use of *Blastocladiella* cDNA microarrays, recently constructed in our laboratory, we intend to analyse the global gene expression of this fungus during its two differentiation phases: the sporulation and the germination. We also intend to investigate *B. emersonii* genes expressed under different nutritional growth conditions. One of the objectives of the project is to characterize cyclin dependent kinases (Cdks) in *B. emersonii*, and to investigate their expression during the sporulation and germination stages. Taking into account preliminary results, we intend to characterize two kinases with non conserved PST AIRE motif (cyclin dependent kinases non PST AIRE) found in *B. emersonii*. In addition, we intend to screen different *B. emersonii* cDNA libraries looking for sequences encoding other putative Cdks for their characterization. Mapping and sequencing of the mitochondrial genome of *Blastocladiella*, aiming a phylogenetic study, will be also carried out during this project.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In *Caulobacter crescentus*, we are investigating the role of ECF sigma factors, which are involved in bacterial responses to distinct types of environmental stresses. The *Caulobacter* genome possesses 13 genes encoding sigma factors of this family. We have up to now characterized 4 of these factors by constructing null mutants of their genes, transcription fusions with their promoters, and through micro array experiments. Our data showed that the ECF sigma factor *SigF* is involved in the response to oxidative stress in stationary phase cells, while *SigT* and *SigU* have a role in osmotic and oxidative stress in the exponential growth phase. Our results also showed that *SigR* is important for the bacterial response to singlet oxygen. Furthermore, we observed that *SigF* is posttranscriptionally controlled, whereas *SigT* and *SigU* are transcriptionally regulated during stress. On the other hand, *SigR* is controlled both transcriptionally and posttranscriptionally.

Caulobacter crescentus *cell cycle*

In *Blastocladiella emersonii*, we are studying differentially expressed genes during the life cycle of the fungus and genes responding to different types of environmental stresses. The construction of cDNA libraries with RNAs isolated from cells at different stages of development or subject to stresses like heat shock and cadmium, followed by large scale sequencing of ESTs, revealed over 6,000 putative distinct genes from this fungus. About 3,700 of these genes were amplified to construct cDNA microarrays which are being used in global transcriptome studies of cells growing under different conditions. Cyclin dependent kinases of the fungus are being also characterized, with the identification of three distinct cDNAs encoding this type of kinases. Analysis of the expression of their genes, associated with determination of their kinase activity during the life cycle is being carried out to uncover their roles in the fungus.

MAIN PUBLICATIONS

Alvarez-Martinez CE, Baldini RL, Gomes SL. 2006. A *Caulobacter crescentus* extracytoplasmic sigma factor mediating the response to oxidative stress in stationary phase. *Journal of Bacteriology.* **188(5)**: 1835-1846.

Susin MF, Baldini RL, Gueiros-Filho F, Gomes SL. 2006. GroES/GroEL and DnaK/DnaJ have distinct roles in stress responses and during cell cycle progression in *Caulobacter crescentus*. *Journal of Bacteriology.* **188(23)**: 8044-8053.

Ribichich KF, Georg RC, Gomes SL. 2006. Comparative EST analysis provides insights into the basal aquatic fungus *Blastocladiella emersonii*. *BMC Genomics* **12**: 177.

Koide T, Salem-Izacc SM, Gomes SL, Vêncio RZN. 2006. SpotWhatR: a user-friendly microarray data analysis system. *Genetics and Molecular Research.* **5(1)**: 93-107.

Vêncio RZN, Koide T, Gomes SL, Pereira CA. 2006. BayGO: Bayesian analysis of ontology term enrichment in microarray data. *BMC Bioinformatics.* **7**: 86.

Georg RC, Gomes SL. 2007. Transcriptome analysis in response to heat shock and cadmium in the aquatic fungus *Blastocladiella emersonii*. *Eukaryotic Cell* **6(6)**:1053-1062, 2007.

Alvarez-Martinez CE, Lourenço RF, Baldini RL, Laub MT, Gomes SL. 2007. The ECF sigma factor ÛT is involved in osmotic and oxidative stress response in *Caulobacter crescentus*. *Molecular Microbiology* **66(5)**: 1240-1255.

Georg RC, Gomes SL. 2007. Comparative expression analysis of members of the Hsp70 family in the chytridiomycete *Blastocladiella emersonii*. *Gene.* **386(1-2)**: 24-34.

Pugliese L, Georg RC, Fietto LG, Gomes SL. 2008. Expression of genes encoding cytosolic and endoplasmic reticulum HSP90 proteins in the aquatic fungus *Blastocladiellla emersonii*. *Gene.* **411(1-2)**: 59-68.

Suely Lopes GOMES

Instituto de Química
Universidade de São Paulo (USP)
Departamento de Bioquímica
Avenida Prof. Lineu Prestes,748 – Butantã
05508-900 – São Paulo, SP – Brasil

+55-11-3091-3826
sulgomes@iq.usp.br