

## BOLSA DE INVESTIGAÇÃO (M/F)

**Referência:** PTDC/BIA-MIC/100370/2008

**Título do Projecto:** “Regulation and maturation of hydrogenases in cyanobacteria”

**Código interno:** PR321707

Está aberto concurso para recrutamento de um(a) bolseiro(a) de Investigação para colaborar no projecto acima referido, financiado pelo programa COMPETE - Programa Operacional Factores de Competitividade na sua componente FEDER e pelo orçamento da Fundação para a Ciência e a Tecnologia na sua componente OE.

A bolsa, em regime de exclusividade, terá a duração de 1 ano eventualmente renovável, com início previsto em 1 de Agosto de 2010.

O valor mensal da bolsa será de € 980,00, pago por transferência bancária (preferencialmente).

**Local de trabalho:** Unidade de Investigação de Microbiologia Celular Aplicada, IBMC, Porto.

**Programa de trabalho:** (ver sumário em anexo).

### **Perfil pretendido:**

Os candidatos devem possuir mestrado na área das Ciências Biológicas, Bioquímica ou afins. Dá-se preferência a candidatos com experiência na área.

O prazo para recepção de candidaturas decorre de 8 a 22 de Julho de 2010.

As propostas deverão incluir CV e uma carta de referência e ser enviadas por correio electrónico para o e-mail [candidaturas@ibmc.up.pt](mailto:candidaturas@ibmc.up.pt) com indicação do código interno (PR321707).

Após avaliação do CV, os candidatos pré-seleccionados poderão ser chamados para entrevista.

A contratação será regida pelo estipulado na legislação em vigor relativamente ao Estatuto de Bolsheiro de Investigação Científica, nomeadamente a Lei 40/2004, de 18 Agosto, e o Regulamento de Bolsas de Investigação Científica do IBMC ([www.ibmc.up.pt/fellowships.php](http://www.ibmc.up.pt/fellowships.php)).

## Regulation and maturation of hydrogenases in cyanobacteria

PTDC/BIA-MIC/100370/2008

### Supervisor

**Paula Tamagnini**

Cellular and Applied Microbiology Unit  
IBMC - Institute for Molecular and Cell Biology  
Rua Campo Alegre, 823  
4150-180 Porto  
Portugal

### **Project Summary:**

Cyanobacteria constitute a large and widespread group of microorganisms that have the ability to perform oxygenic photosynthesis and some can fix atmospheric nitrogen. Cyanobacteria may possess up to two hydrogenases. The nitrogen-fixing strains, with the exception of some *Synechococcus*, possess an uptake hydrogenase that catalyzes the consumption of the H<sub>2</sub> produced by the nitrogenase during the reduction of N<sub>2</sub> to NH<sub>3</sub>. Additionally, they may harbor a bidirectional hydrogenase, an enzyme that is generally present in non-N<sub>2</sub>-fixing strains. Both cyanobacterial hydrogenases are metalloproteins containing Ni and Fe in their active sites. The biosynthesis/maturation of NiFe-hydrogenases is a highly complex process requiring several proteins for the incorporation of the metal ions as well as CO and CN ligands in the active centre (large subunit), the insertion of the FeS clusters (small subunit), and the cleavage of a C-terminal polypeptide as the final step in the maturation of the large subunit. Subsequently, the mature large subunit can be assembled with the mature small subunit forming the functional enzyme. The genes/proteins related to the maturation of hydrogenases, and the process itself, were firstly characterized for *Escherichia coli*, and while most of the Hyp proteins affect hydrogenases pleiotropically, the large subunit of each hydrogenase is proteolytically processed by a specific endopeptidase. The uptake and bidirectional hydrogenases have been characterized in different cyanobacteria, yet the knowledge on their biosynthesis/maturation process is still scarce. To date, several genes presumably involved in these processes have been identified, clustered or scattered throughout the genomes of several cyanobacterial strains. The presence of a single copy of most of the *hyp* genes in the genome of cyanobacteria, regardless of possessing either one or both hydrogenases, suggests that the Hyp proteins might be responsible for the maturation of both hydrogenases. The genes encoding the putative cyanobacterial specific C-terminal endopeptidases (*hupW* and *boxW*) were also identified by analyzing the available genome sequences. The maturation of the small subunit of NiFe-hydrogenases is poorly understood and there are only *in silico* studies regarding this process in cyanobacteria. It was suggested that proteins encoded by ORFs in the vicinity of *hyp* genes could be implicated in the maturation of the uptake hydrogenase small subunit, notably inserting the FeS clusters that function as electron transfer domains. Analysis of the promoter regions of cyanobacterial *hyp* genes revealed that the two transcriptional regulators NtcA and LexA - recently implicated in the regulation of cyanobacterial uptake and bidirectional hydrogenases structural genes, respectively - could also be involved in the transcriptional regulation of the genes encoding proteins responsible for their maturation. The only work directly addressing the involvement of the Hyp proteins and HoxW (one of the endopeptidases) in cyanobacterial hydrogenase maturation process was performed in *Synechocystis* sp. PCC 6803. In this organism, the inactivation of each of the *hyp* genes and *boxW* lead to the loss of the bidirectional hydrogenase activity. However, *Synechocystis* sp. PCC 6803 harbors only the bidirectional hydrogenase, and therefore studies on strains containing only the uptake or both enzymes are required. For this project the filamentous heterocystous *Nostoc* sp. PCC 7120 was chosen as model organism. This strain contains the uptake and the bidirectional hydrogenase, its genome sequence and a variety of molecular tools for genetic manipulation are available. Moreover, several promoter regions upstream of hydrogenases related genes have been identified and analyzed for this organism, revealing putative binding sites for the transcriptional regulators LexA (in *box* promoters), and NtcA (in *hyp* promoters). Furthermore, *Nostoc* sp. PCC 7120 *hup*, *hox*, *hup*/*hox* mutants have already been constructed and are currently at our disposal through a Material Transfer Agreement with Waseda University (Japan). The major aims of this project are: (i) demonstrate the involvement of the Hyp proteins in the biosynthesis/maturation of both hydrogenases – particularly in the uptake hydrogenase; (ii) prove the involvement and specificity of the endopeptidases, HoxW and HupW, in the cleavage of the C-terminal polypeptide of each hydrogenase large subunit precursor; (iii) investigate the contribution of other proteins to the maturation process, notably products of ORFs in the vicinity of *hyp* genes; and (iv) identify other transcription factors implicated in the regulation of hydrogenases related genes.

This work will generate new knowledge that will contribute to understand the mechanisms of regulation and maturation of cyanobacterial hydrogenases, and will allow in the future the optimization of photobiological H<sub>2</sub> production by these organisms.