

Cellular networks linked to protein synthesis and human disease



Luís Ribas de Pouplana

Our laboratory studies the connections between the protein synthesis machinery and the rest of the cellular environment. Gene expression and protein synthesis constitute the core of molecular biology and are therefore intimately linked to all the networks that regulate cells and tissues. The sophisticated machinery required to translate genes is well characterised, but the integration of genetic code components with the rest of the cellular metabolism is poorly understood. Alterations in the delicate balance that links protein synthesis to cell biology cause a vast array of illnesses. Elucidation of this balance is essential for the development of new therapeutic agents. Our research efforts focus on studying the associations between gene expression, protein synthesis and human disease. The projects undertaken in our laboratory can be divided into three general fields: gene expression and parasite pathogenicity, organelle protein expression and mitochondrial disease, and protein synthesis disorders and drug discovery.

Gene expression and parasite pathogenicity (Manuel Castro, Alfred Cortes, Valerie Crowley and Thomas Jones)

Our research addresses the relationship between infectious processes in humans and the protein synthesis apparatus of the pathogens involved. Thomas Jones is currently characterising an unusual aminoacyl-tRNA synthetase in the human intracellular parasite *Mycoplasma penetrans*. This parasite presents

remarkable genome reduction and yet some of its protein synthesis components display new domains of unknown function. The study of this protein has revealed a new mechanism for the discrimination of methionine and isoleucine during protein synthesis (Jones *et al*, 2008; Figure 1).

Our studies also deal with genome dynamics and the functional role of a family of inflammation-activator domains that are transferred between enzymes involved in protein synthesis through a ubiquitous process of domain shuffling, which remains to be elucidated. In the human pathogen *Entamoeba histolytica*, one of these domains doubles its genomic dose through its selective integration into two unrelated enzymes. We have shown that *Entamoeba* generates a functional mimic of a human pro-inflammatory cytokine, and we are now examining its role in infection.

Our research effort also focuses on the contribution of gene regulation to the infection of red blood cells by the parasite *Plasmodium falciparum*, which is the main causal agent of malaria and responsible for over a million deaths a year. Using a multigenic family of proteins that display differential expression in clonal lines of *P. falciparum*, we study the role of chromatin structure and modification in the control of gene expression in *Plasmodium*. This research is directed by Alfred Cortes (Cortés *et al*, 2007).

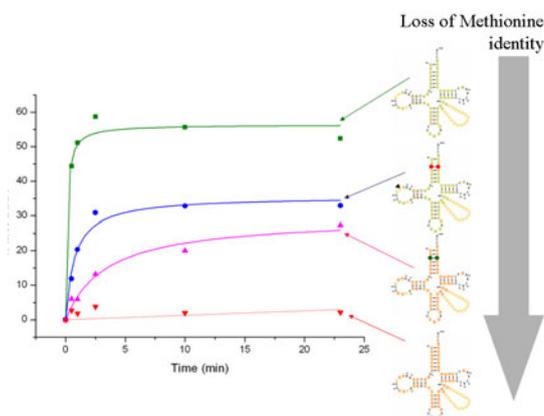
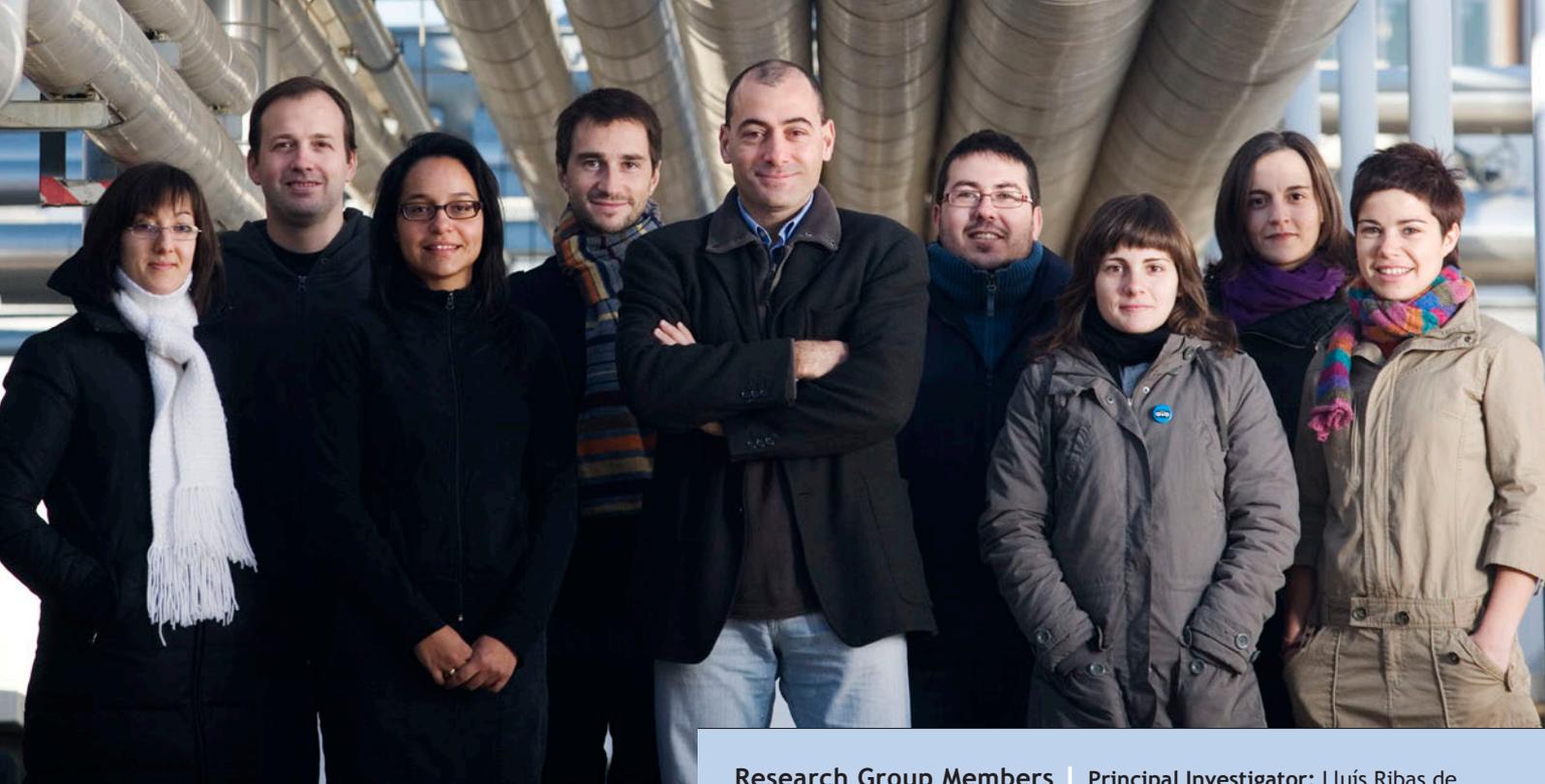


Figure 1. Manipulation of the methionine identity of *Mycoplasma penetrans* tRNAMet following new rules for methionine and isoleucine discrimination.



Research Group Members | Principal Investigator: Lluís Ribas de Pouplana | Associate Researcher: Alfred Cortés Closas | Postdoctoral Fellow: Renaud Geslain | PhD Students: Manuel Castro de Moura, Valerie Crowley, Yaiza Español Fernández, Tanit Guitart Rodés, Thomas Jones | Lab Technician: Noelia Camacho Hinojosa | Master Student: Ana Bernardó | Visitors: Ana Catarina Gomes (Portugal), Assitan Sidibe (France)

Organelle protein expression and mitochondrial disease

(Yaiza Español and Tanit Guitart)

In this line of research, we examine the cellular mechanisms that control and coordinate protein synthesis in the lumen of cellular organelles, such as mitochondria. In humans, several forms of muscle-wasting diseases are caused by defects in mitochondrial protein synthesis machinery. The multi-clonal nature of mitochondria makes the study of these conditions extremely difficult. A long-term goal of our laboratory is to generate an animal model for mitochondrial disease in order to facilitate research into muscle-wasting disorders.

To this end, we have started characterising the mitochondrial protein synthesis machinery in *Drosophila*. Tanit Guitart is studying the seryl-tRNA synthetases of this organism and developing strains of *Drosophila* in which the expression of mitochondrial synthetases is experimentally repressible, in order to generate flies that reproduce the defects in mitochondrial protein synthesis that cause muscle-wasting in humans (Figure 2).

Most of the proteins required for protein synthesis in the mitochondria are nuclear-encoded, and are imported into this organelle. In contrast, most species have maintained their mitochondrial tRNA genes in the mitochondrial genome. Interestingly, several mitochondrial tRNA aminoacylation enzymes are organelle-specific. *Trypanosoma* represents an extreme case because their mitochondrial genomes code for only 4 tRNAs but the mitochondria maintains several specific nuclear-encoded aminoacyl-tRNA synthetases. What prevents the eukaryotic cell from reducing the complexity of its protein synthesis apparatus and using the same aminoacyl-tRNA synthetases (ARS) in

the nucleus and the mitochondria? To answer this question, Yaiza Español is currently characterising the lysyl-tRNA synthetase system in *Trypanosoma brucei*.

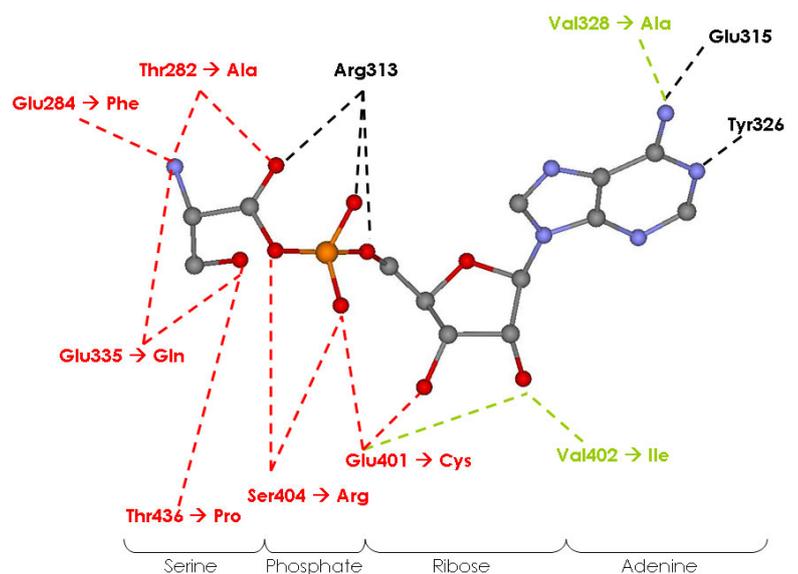


Figure 2. Structural analysis of the catalytic centre of a *Drosophila melanogaster* mitochondrial seryl-tRNA synthetase in relation to the binding of the enzyme's reaction intermediate.

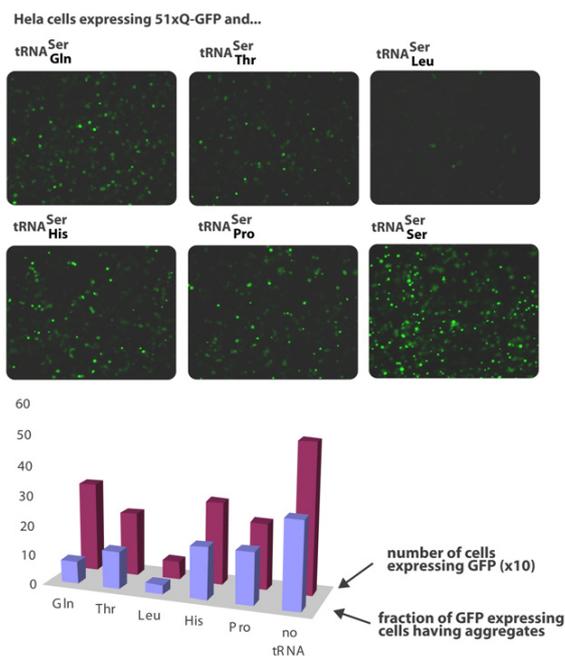


Figure 3. Analysis of the effect of generalised protein translation errors caused by mutant tRNAs and monitored through the detection of GFP fluorescence.

Protein synthesis disorders and drug discovery

(Noelia Camacho and Renaud Geslain)

Laboratory efforts to develop a new screening procedure for molecular inhibitors of aminoacyl-tRNA synthetases have resulted in the creation of a spin-off company, Omnia Molecular, which will continue to develop this project commercially. This company is hosted by the bioincubator of the Barcelona Science Park, and has six employees.

We continue to study the effects of protein synthesis errors in human cells. Renaud Geslain is addressing the stress mechanisms that respond to gross misacylation of tRNAs to minimize the deleterious effect of generalised errors in protein synthesis (Figure 3). In parallel, we continue our collaboration with the Combinatorial Chemistry Laboratory of the Barcelona Science Park, which provides us with *ad hoc* chemical libraries designed to bind and inhibit the active sites of aminoacyl-tRNA synthetases. Noelia Camacho, in collaboration with Dr. Cortes, is testing these compounds in *Plasmodium*, and characterising the effect that protein synthesis inhibitors have on the infection of red blood cells by the malaria parasite (Farrera *et al.*, in preparation).

Publications

Cifuentes D, Martínez-Pons C, García-Rocha M, Galina A, Ribas de Pouplana L and Guinovart J. Hepatic glycogen synthesis in the absence of GK. The case of embryonic liver. *J Biol Chem*, **283**(9), 5642-49 (2008); Epub Dec 28 (2007)

Cortés C, Carret O, Kaneko BYS, Yim Lim A, Ivens A and Holder AA. Epigenetic silencing of *Plasmodium falciparum* genes linked to erythrocyte invasion. *PLoS Pathog*, **3**, e107 (2007)

Jones TE, Brown CL, Geslain R, Alexander, RW and Ribas de Pouplana L. An operational RNA code for faithful assignment of AUG triplets to methionine. *Mol Cell*, **29**(3), 401-07 (2008)

Research Networks and Grants

Desarrollo de un nuevo método para la selección de antibióticos

Spanish Ministry of Science and Education, BIO2006-01558: 2007-2009

Research Director: Lluís Ribas de Pouplana

Mecanismos de silenciamiento de genes de Plasmodium falciparum que codifican ligandos para la invasión de eritrocitos y fenotipos asociados al silenciamiento o activación de los mismos

Spanish Ministry of Health, PI070891: 2008-2010

Research Director: Alfred Cortés i Closas

Support to exceptional research groups

Generalitat de Catalunya, SGR00350: 2006-2008

Research Director: Lluís Ribas de Pouplana

Collaborations

Combinatorial libraries of aminoacyl-adenylate analogs
Miriam Royo, Combinatorial Chemistry Unit, Barcelona Science Park (Barcelona, Spain)

Development of positive selection screens for antibiotic discovery
Omnia Molecular SL (Barcelona, Spain)

Expression strategies for Plasmodium proteins
ERA Plantech SL (Barcelona, Spain)

Functional evolution of the glycogen metabolism
Daniel Cifuentes and Joan Guinovart, IRB Barcelona (Barcelona, Spain)

Inflammatory effect of an Entamoeba histolytica MetRS domain
Annabel Valledor and Antonio Celada, IRB Barcelona (Barcelona, Spain)

Methionine metabolism and pathogenicity in the human pathogen Mycoplasma penetrans
Rebecca Alexander, Wake Forest University (NC, USA)

Mitochondrial protein synthesis in Drosophila melanogaster development
Thomas Stratmann, University of Barcelona (Barcelona, Spain)