



Understanding the connections between protein synthesis and disease

The machinery of protein synthesis is a central part of the cell and is in many ways linked to a number of human diseases. Our goal is to contribute to the understanding of genetic code biology and its integration within cellular metabolism. In recent years it has become evident that aminoacyl-tRNA synthetases (ARSs) play key roles in cell cycle regulation, the control of gene expression, cell-cell communication and tissue development. This array of new functions has directly linked ARSs and protein synthesis with regulatory cellular pathways and an increasing number of human diseases.

Study of the response mechanisms to proteomic errors in human cells

Misfolded proteins are caused by genomic mutations, aberrant splicing events, translation errors, and environmental factors. The accumulation of misfolded proteins is a phenomenon connected to several human disorders and is managed by stress responses specific to the cellular compartments affected. In wild-type cells mechanisms of stress response can be experimentally induced by expressing recombinant misfolded proteins or by incubating cells with large concentrations of amino acid analogues. We have developed a novel approach for the induction of stress responses to protein aggregation. Our method is based on engineered transfer RNAs (tRNAs) that can be expressed in cells or tissues, where they actively integrate in the translational machinery and cause general proteome substitutions.

We have engineered a battery of mutagenic tRNAs that introduce a range of ten distinct mutations in a human cell type and in a vertebrate embryonic model (in collaboration with Elisa Martí, IBMC-CSIC, and David Rossell, IRB Barcelona). In order to rapidly follow the effect of each tRNA, we have constructed a GFP protein that is not affected by the mutagenic tRNAs and that can be used as a marker of the overall physiological state of the cells. This method allows for the controlled induction of generalised proteome defects in a direct manner, without the potential for other secondary effects. This strategy also permits the uniform introduction of different types of mutations throughout the proteome, and can be applied to the analysis of the timing and grade of stress responses, as well as to the identification of new links between these responses (Figure 1).

This strategy allows the random introduction of mutations of increasing severity in the proteome, without exposing cells to unnatural compounds. We have shown that this approach can be used for the differential activation of the stress response in the endoplasmic reticulum. As an example of the applications of this method, we have applied it to the identification of human

microRNAs activated or repressed during unfolded protein stress (Geslain *et al*, 2009).

Generation of an animal model for mitochondrial disease linked to translation defects

The mitochondrial tRNA serylation system is relevant in biomedical terms because mutations in human tRNA^{Ser} cause mitochondrial encephalomyopathy and lactic acidosis (MELAS). MELAS constitutes a loose family of mitochondrial diseases often produced

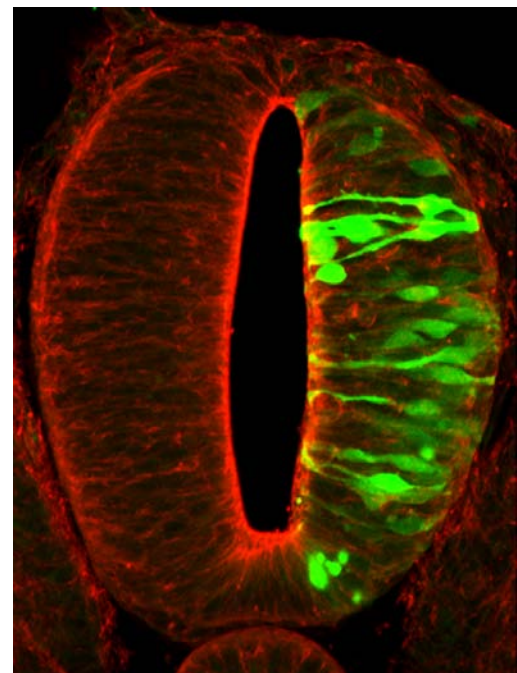


Figure 1. Expression of heterologous tRNAs in a chicken embryo model.

by mutation in mitochondrial tRNA genes and, occasionally, by mutations in nuclear-encoded mitochondrial ARS. The nature of mitochondria makes these disorders extremely difficult to study and this justifies the search for animal models that facilitate the characterisation of these illnesses and the search for palliative measures for their symptoms.

Seryl-tRNA synthetases (SRSs) are the enzymes responsible for the serylation of tRNA^{Ser}. SRSs are dimeric enzymes that belong to the subclass IIa of aminoacyl-tRNA synthetases (ARSs). In metazoans, SRSs are among the few enzymes that remain duplicated in the cell, one isoform acts in the cytosol and the second functions in the mitochondria, where it recognises the highly diverged structures of mitochondrial tRNA^{Ser}. During the process of building a model for human deficiency in mitochondrial tRNA^{Ser} aminoacylation in *Drosophila melanogaster*, we realised that the *D. melanogaster* genome contains three genes coding for SRS. All three proteins coded by these genes contain the canonical class II ARS motifs, and share a significant level of sequence identity among them. In order to identify the mitochondrial SRS in *Drosophila*, we are characterising the products of these three genes.

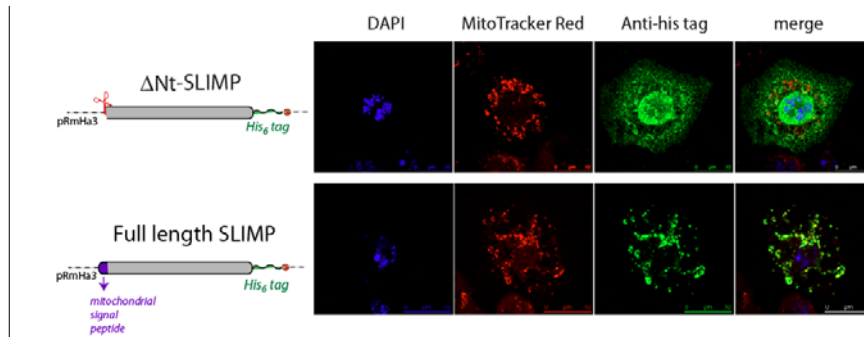


Figure 2. Cellular distribution of SLIMP.

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During 2009 we have functionally characterised one of these proteins, provisionally named SLIMP (SRS-Like Insect Mitochondrial Protein). This polypeptide is not a functional SRS. However, the gene coding for SLIMP is universally present in the insect genomes available, and the protein is expressed in species of diptera and coleoptera. SLIMP expression is developmentally regulated in *D. melanogaster*, and the protein is localised to the mitochondria through a signal peptide that is processed upon translocation.

The function of the protein is essential to *D. melanogaster* as repression of its expression by RNAi treatment is lethal to the animals. However, this lethal effect can be strongly suppressed by supplementing the flies' diet with known anti-oxidant molecules. This observation suggests that depletion of the protein causes oxidative stress in the affected animals (Guitart *et al*, in preparation; Figure 2).

Studying the biology of ARSs in human parasites

ARSs are multi-domain proteins responsible for the aminoacylation of tRNAs. Throughout the phylogenetic tree, ARSs and their related proteins carry out additional cellular functions and may be implicated in other metabolic pathways, cell signalling mechanisms, and developmental processes. The protein synthesis machinery represents one of the most useful targets for the development of new anti-infectives. Several widely used antibiotics exert their function by blocking the protein synthesis machinery.

However, very little is known about the specifics of the protein synthesis machinery in parasites. This lack of information about this key metabolic pathway in parasites clearly hinders the possibility of transferring knowledge in protein synthesis to the development of new drugs directed against the translational machinery of these organisms. We aim to contribute to closing this knowledge gap through the analysis of ARSs in human parasites of the genera *Plasmodium* (the causal agents of malaria), *Entamoeba* (causal agent of amoebic dysentery) and *Trypanosoma* (causal agents of Chaga's disease; Español *et al*, 2009; Novoa *et al*, in press; Castro *et al*, in preparation; Krog *et al*, in preparation).

In eukaryotes, it is well established that cytoplasmic ARSs form multi-enzyme complexes composed of up to eleven individual ARSs. These complexes are structurally stable and assemble around three additional proteins (AIMP1, AIMP2, and AIMP3), which are essential for the formation of the complex and also act as cytokines. Little is known about additional functions of ARS in protozoan parasites, but we are studying a potential example of such functions in the protozoan *Entamoeba histolytica*.

E. histolytica, an amitochondriate unicellular protozoa, is the leading cause of human death by dysentery worldwide. The main clinical complications caused by *E. histolytica* infections are due to the capacity of the parasite to traverse the intestinal wall and infect the internal organs of its host. Protozoan human parasites have evolved a variety of strategies to respond and modulate the immune responses of their host. We have discovered that several *Entamoeba* ARS genes contain a C-terminal domain homologous to the pro-inflammatory human AIMP1 cytokine. In collaboration with Antonio Celada's group (IRB Barcelona), we are characteris-

ing the biological role of the AIMP1-like domains in *Entamoeba*.

We have shown that the purified AIMP1-like domain that is coded by *Entamoeba* ARS genes displays significant dose-dependent cytokine activity in mammalian cell signalling assays, at concentrations comparable to those required by human AIMP1 to obtain similar effects. The protein is located in cell periphery and is readily cleaved by *Entamoeba* proteases to release the AIMP1-like domain. Our current hypothesis is that this protein is involved in the formation of liver granulomas during the systemic infection sometimes caused by *E. histolytica* in humans (Figure 3).

Identification of new anti-malarial molecules that target Plasmodium falciparum ARS

ARSs are essential enzymes that constitute well-known targets for antibacterial compounds (including the commercial antibiotic BACTRABAM); however, they have not been explored in the search for anti-malarial drugs. The goal of this project is to study the effect of known ARS inhibitors on the *Plasmodium* life cycle, to determine their effectiveness and specificity, and to use these data to identify target enzymes and chemical scaffolds of interest for further chemical design. This project forms part of *Mephitis*, a European consortium funded under FP7 and coordinated by our laboratory.

We have assembled an initial battery of known ARS inhibitors and tested them for their capacity to reduce the growth rate of *P. falciparum* in human blood. In initial tests, we have observed three inhibitory effects. After addition of drugs, borrelidin and the amino acid adenylate analogues showed an immediate effect on *P. falciparum* at pharmacologically relevant concentrations. Pseudomonic acid, as reported for other inhibitors of the protein synthesis machinery, showed a delayed effect that is evident at 96 hours of culture. This observation suggests that

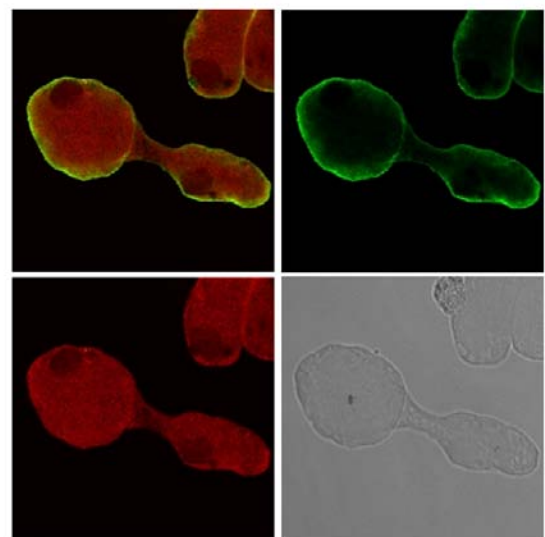


Figure 3. Distribution of AIMP1 (green) in *Entamoeba histolytica*.

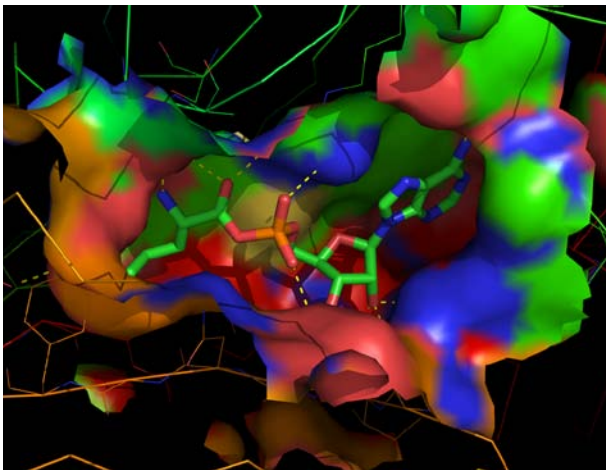


Figure 4. Three-dimensional model of a Plasmodium ARS active site.

the drug specifically inhibits the apicoplastic ARS, but not its cytosolic counterpart.

We have analysed the effect of all the components of our chemical battery on human cells and bacteria to determine their species specificity. The tests performed indicate that most of the compounds tested are highly toxic to HeLa cells. However, pseudomonic acid and borrelidin do not affect the growth of human cells despite being effective anti-bacterials. These two compounds have been tested in an animal model of *Plasmodium* infection (in collaboration with José Manuel Bautista, UCM, Madrid). Remarkably, borrelidin is as active as the anti-malarial drug chloroquine in protecting mice from malaria (Camacho *et al*, in preparation). We are characterising the behaviour of this molecule and of chemical analogues. In parallel, we have started a computational drug design project to obtain dual-inhibitors of *Plasmodium* ARS. This is a collaboration project with the Combinatorial Chemistry Laboratory, managed by Miriam Royo at the Barcelona Science Park (Figure 4).

Scientific output

Publications

Español Y, Thut D, Schneider A and Ribas de Pouplana L. A mechanism for functional segregation of mitochondrial and cytosolic genetic codes. *Proc Natl Acad Sci USA*, **106**(46), 19240-45 (2009)

Geslain R, Cubells R, Bori-Sanz T, Álvarez-Medina R, Rossell D, Martí E and Ribas de Pouplana L. Chimeric tRNAs as tools to induce proteome damage and identify components of stress responses. *Nucleic Acids Res*, Epub Dec 8 (2009)

Research networks and grants

Desarrollo de un nuevo método para la selección de antibióticos
Spanish Ministry of Science and Innovation, BIO2006-01558 (2007-2009)

Principal investigator: Lluís Ribas de Pouplana

Implicación de componentes del código genético en patologías humanas

Spanish Ministry of Science and Innovation, BIO2009-09776 (2009-2012)

Principal investigator: 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

Carlos III Health Institute (ISCIII), BIO2006-01558 (2007-2010)
Researcher: Alfred Cortés

Targeting protein synthesis in the apicoplast and cytoplasm of Plasmodium (Mephitis)

European Commission, FP7-HEALTH-223024 (2009-2011)

Principal investigator and coordinator: Lluís Ribas de Pouplana

Collaborations

Analysis of protein mistranslation in a vertebrate model
Elisa Martí, IBMC-CSIC (Barcelona, Spain)

Characterisation of antimalarial activities of ARS inhibitors
José Manuel Bautista, UCM Madrid (Madrid, Spain)

Characterisation of Mycoplasma penetrans MetRS
Rebecca Alexander, Wake Forest University (Winston-Salem, USA)

Construction of a Plasmodium KO for PfTRBP111
Mafgali Frugier, IBMC-CNRS (Strasbourg, France)

Design of new dual ARS inhibitors
Miriam Royo, Barcelona Science Park (Barcelona, Spain)

Role of AIMP1 in Entamoeba infections
Antonio Celada, IRB Barcelona (Barcelona, Spain)

Role of AIMP1 in Entamoeba infections
Sunghoon Kim, Seoul University (Seoul, Korea)

Statistical analysis of protein mistranslation in eukaryotic cells
David Rossell, IRB Barcelona (Barcelona, Spain)