LLUÍS RIBAS DE POUPLANA

Gene translation laboratory

The gene translation machinery is directly linked to a large number of human diseases. Our general goal is to contribute to the understanding of genetic code biology and its integration within the cellular metabolism. In recent years it has become evident that aminoacyl-tRNA synthetases (ARSs) play central 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. Our current projects and aims are as follows: (i) to study the effect of the accumulation of translation errors on human cells, and identify the mechanisms of control and correction of such problems; (ii) to generate an animal model for mitochondrial disease linked to translation defects and to study the cellular responses to increasing levels of protein synthesis disruption; (iii) to study the role of cytokine-like ARS domains in the infectious cycle of *Entamoeba*; and (iv) to examine the set of ARSs in *Plasmodium falciparum* and identify new inhibitors of these enzymes.

The effect of the accumulation of translation errors on human cells, and identification of the mechanisms of control and correction of such problems

The human genome codes for at least 30,000 proteins, and the machinery required to produce these makes, on average, one error per 1,000 amino acids that are incorporated into polypeptide chains (Edelmann and Gallant, 1977). The capacity of ARSs to specifically recognise their cognate amino acid and tRNA substrates and, in some cases, to correct initial recognition errors via their editing domains, constitutes the main proofreading step in protein synthesis. A complementary check is later performed by elongation factors, which can reject certain misacylated tRNAs (LaRiviere *et al*, 2001).

A decrease in the fidelity levels of the protein synthesis apparatus has been shown to be deleterious and potentially lethal. Several studies have demonstrated that an increase in the rate of error during protein synthesis reduces cell viability (Beebe *et al*, 2003). In mammals, the malfunctioning of some elements of the translation machinery causes disease. For example, a point mutation in the editing domain of alanine-tRNA synthetase (AlaRS) causes cerebellar Purkinje cell loss and ataxia in mice (Lee *et al*, 2006). This mutant AlaRS has lost its capacity to hydrolyse ser-tRNAAla.

We have devised a genetic approach with engineered tRNAs to introduce random and widespread mutations of increasing impact into a metazoan proteome. Using a human cell line and an embryonic vertebrate model, we have studied the cellular reaction to increasing amounts of mutagenic insult and have identified the pathways that react first, and proportionally, to the accumulation of proteome defects. Our results indicate that the endoplasmatic reticulum acts, through the activation of the unfolded protein response (UPR), as the cellular sensor for overall protein quality, reacting steadily to the accumulation of errors that escape the action of upstream editing mechanisms.

We have shown that the UPR acts not only as a regulator of the quality of secreted proteins, but also as a general sensor for the condition of the entire cellular proteome. The UPR reacts earlier than the ubiquitination pathway to errors in protein synthesis and, unlike the proteasome, it is activated differentially depending on the degree of biophysical stress caused by the errors in translation (Geslain *et al*, in preparation).

Comparable to the effect of a mischarging ARS would be the advent of widespread mutations in the genome. It has been amply reported that genomic mutational damage caused by radiation or exposure to DNA-modifying chemicals can cause cell death. However, very little is known about the control mechanisms used by cells to respond to mistranslation events that escape the control mechanisms aforementioned. Consequently, this project aims to characterise, in a quantitative manner, the cellular response to this kind of insult.

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Generating a model for mitochondrial disease linked to translation defects and studying the cellular responses to increasing levels of protein synthesis disruption

The mitochondrial translational machinery is responsible for the synthesis of the respiratory chain proteins encoded by the organelle genome. Therefore, defects in the mitochondrial translational apparatus lead to a variety of health disorders, mainly related to mitochondrial metabolism.

More than 100 mutations involving rRNA and tRNA have been found in the human mtDNA. Furthermore, several diseases have been reported to be related to mutations in mt-tRNA modification proteins or in mitochondrial proteins involved in translation. In the last few years a number of mitochondrial ARS mutations have been found to cause central nervous system disorders (Scheper *et al*, 2007; Edvarson *et al*, 2007) or shown to be susceptibility agents for diabetes mellitus ('t Hart *et al*, 2005). It is generally believed that mitochondrial diseases caused by mutations in ARS and tRNAs are caused by the effect of these mutations on the protein synthesis rate of the organelle.

A characteristic of mitochondrial metabolic diseases is their extreme case-to-case variability, a feature attributed to the polyclonal nature of mitochondria. This feature, together with the experimental difficulty of manipulating mitochondria, makes these diseases extremely refractive to research efforts. Thus, the complexity and medical relevance of mitochondrial diseases justifies interest in developing animal models to study the consequences of mitochondrial aminoacylation deficiencies.

In this line of research, we are building an animal model for mitochondrial disease in *Drosophila melanogaster* by generating tissue-specific and gradual deficiencies in mitochondrial serylation in this organism. To achieve this goal, we are generating fly strains in which the nuclear-encoded mitochondrial seryl-tRNA synthetase (DmSRS2) can be silenced by interference RNA (Brand *et al*, 1993). We have observed that the ubiquitous and constitutive silencing of DmSRS2 produces lethality and that tissue-specific silencing leads to a range of phenotypic defects in the fly. We are currently evaluating these defects and studying the stress pathways activated by this insult (Figure 1).

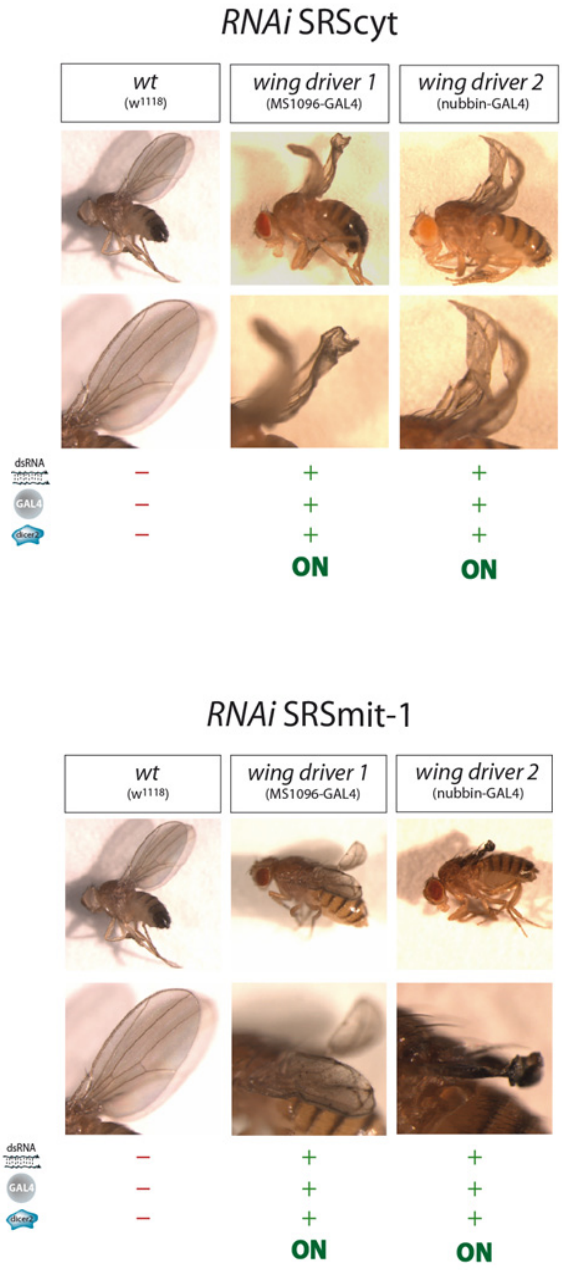

Figure 1. *Effect of silencing of cytosolic (top) and mitochondrial (bottom) SRSs in the Drosophila wing.*

Studying the role of cytokine-like ARS domains in the infectious cycle of *Entamoeba*

ARSs and related proteins carry out additional cellular functions and are implicated in several metabolic pathways, cell signalling mechanisms, and developmental processes (Ribas de Pouplana and Geslain, 2008). AIMP1, the best characterised of these proteins, is a wide acting cytokine that induces inflammatory responses in endothelial cells, apoptosis, and migration of inflammation-related cell types. AIMP1 and derived peptides are active in a large number of cell signalling and developmental pathways (van Horssen *et al*, 2006).

Protozoan parasites have evolved a variety of strategies to evade the immune responses of their hosts. In the best documented cases, these adaptations involve the modification of the parasite's surface to escape the immune response (Cortes, 2007). We have discovered that in several *Entamoeba* species the genes coding for methionyl- and lysyl-tRNA synthetases (EhKRS) contain a C-terminal domain homologous to the human AIMP1 cytokine. *Entamoeba* is the first case in which this domain is found attached to KRS, and it is also the first known case of a genome containing two copies of human-like AIMP1-type domains attached to distinct genes. In this project, we explore the interactions between mammalian inflammatory pathways and the human-like cytokine (EhAIMP) that we have recently discovered in the genus *Entamoeba*.

Kinetic characterisation of the protein showed that the functional role of EhAIMP1 is not related to the aminoacylation reaction of $tRNA^{Lys}$. To test whether EhAIMP1 was capable of mimicking the functions of human AIMP1, we checked its capacity to affect the viability of bovine aorta endothelial cells (BAECs). We showed that EhAIMP1 and EhKRS induced apoptosis in BAECs in a dose-dependent manner at concentrations comparable to those required to exert the same effect with human AIMP1.

We also analysed the TNF-α secretion level after the addition of EhKRS, EhKRSΔ and EhAIMP1 proteins and observed an increase in TNFα release to similar levels as those caused by human AIMP1 (Figure 2). This effect is mainly due to the activity of the EhKRS synthetase domain, although the EhAIMP1 domain also produces a small effect. Inactivated *Entamoeba* proteins do not produce an increase in TNFα secretion. These results support the notion that this protein mimics the role of its human counterpart and that it participates in the interaction of *Entamoeba* with its human hosts during the infection cycle.

Studying the set of ARSs in *P. falciparum* and identifying new inhibitors of these enzymes

This research project is focused on tRNA biology in *P. falciparum* and on the development of a new pharmacological screen for the discovery of *Plasmodium* ARS inhibitors. Protein synthesis in *Plasmodium* remains largely uncharacterised. As an example, the first experimental evidence that protein translation is active in *Plasmodium* apicoplasts was obtained as recently as 2005 (Chaubey *et al*, 2005). Furthermore, it has long been recognised that protein synthesis inhibitors, like doxycycline, are active against *P. falciparum* and are useful drugs in the treatment of malaria (Clyde *et al*, 1971). Thus, the study of components of

the genetic code of *P. falciparum* may provide new and relevant information on the biology of the parasite and, more importantly, may open up new leads for the development of novel anti-malarials.

Genomic data readily allows the identification of the complete set of *P. falciparum* ARS genes, including several duplicated ones that could correspond to enzymes that are active in the apicoplast or the mitochondria of the parasite. At present, the cellular localisation of several of these enzymes is unclear, particularly in the mitochondria where the predicted set of ARS is clearly incomplete.

Preliminary analysis of several *P. falciparum* life cycle inhibitors performed in our lab has shown several types of inhibitory effects, characterised in some cases by a delayed mode of action (Camacho *et al*, in preparation). More specifically, up to three types of inhibitors can be identified on the basis of the delay observed before arrest of the erythrocyte invasion cycle. These delayed effects have been previously linked to disruptions of the apicoplast metabolism (Dahl *et al*, 2007).

A central focus of this research line lies in drug development. Particularly interesting as drug targets are ARSs that are unique to *P. falciparum* and not present in human cells. In this project, we plan to analyse known inhibitors of ARSs for their potential use against *P. falciparum*, construct combinatorial chemistry libraries based on the molecular data obtained by several of the partners involved, and develop a new method for the discovery of new inhibitors based on a positive selection scheme that will accelerate the current methods of drug discovery. In this regard, our lab coordinates an EU consortium of eight laboratories that seeks to elucidate several aspects of gene translation in this parasite and extend this knowledge to the discovery of new inhibitory molecules.

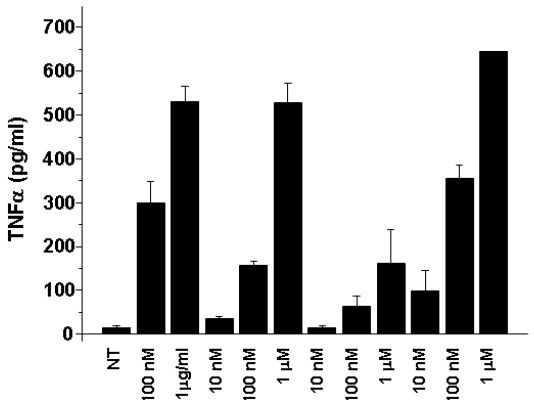

Figure 2. *EhAIMP1 causes an increase in TNFα secretion in human RAW264.7 cells.*

SCIENTIFIC OUTPUT

Publications

Cifuentes D, Martínez-Pons C, García-Rocha M, Galina A, de Pouplana LR and Guinovart JJ. Hepatic glycogen synthesis in the absence of glucokinase: the case of embryonic liver. *J Biol Chem*, **283**(9), 5642-49 (2008)

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Research networks and grants

Ayuda para la preparación del proyecto europeo 'Planificación de transmal: una red europea de investigación sobre malaria'
Spanish Ministry of Science and Innovation, SAF2007-29381-E (2008)
Principal investigator: Lluís Ribas de Pouplana

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

Plasmodium falciparum que codifican ligandos para la invasión de eritrocitos y fenotipos asociados al silenciamiento o activación de los mismos
Instituto de Salud Carlos III, PI07891 (2007-2010)
Principal investigator: Lluís Ribas de Pouplana

Support to exceptional research groups
Agency for Administration of University and Research Grants (AGAUR), 2005-SGR-00350 (2006-2008)
Principal investigator: Lluís Ribas de Pouplana

Targeting protein synthesis in the apicoplast and cytoplasm of plasmodium (MEPHITIS)
European Commission, FP7-HEALTH-2007-B (2008-2011)
Principal investigator: Lluís Ribas de Pouplana (coordinator)

Collaborations

Characterisation of Entamoeba EhAIMP1
Sunghoon Kim, University of Seoul (Seoul, Korea)

Characterisation of Entamoeba EhAIMP1
Antonio Celada, IRB Barcelona (Barcelona, Spain)

Characterisation of Mycoplasma penetrans LysRS
Rebecca Alexander, Wake Forest University (North Carolina, USA)

Discovery of ARS inhibitors
Omnia Molecular SL (Barcelona, Spain)

Evolution of human ARS domains
aTyr Pharma (San Diego, USA)

Generation of animal models of ARS-related diseases
Elena Jordanova, University of Antwerp (Antwerp, Belgium)

Generation of an animal model of cellular mistranslation
Elisa Martí, Institute of Molecular Biology of Barcelona, Spanish National Research Council (IBMB-CSIC) (Barcelona, Spain)

Protein synthesis in Plasmodium
EU-funded consortium of eight laboratories coordinated by our group