Exploring the cellular network linked to protein synthesis

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We study the connections between the protein synthesis machinery and the rest of the cellular environment, mainly in human protozoan parasites (Figure 1). Gene expression and protein synthesis constitute the central core of molecular biology and, as such, are tightly linked to the networks that regulate cells and tissues. The sophisticated machinery required to translate genes is now well characterised, and this body of knowledge offers the possibility to investigate how the components for genetic code translation influence and integrate themselves with the rest of the cellular metabolism. We focus mainly on the links between gene expression and pathogenicity in human pathogens. Our activities also address organelle-specific protein synthesis and the evolution of the eukaryotic cell through the analysis of gene translation apparatus.

Gene expression and parasite pathogenicity

We examine the connections between infectious processes in humans and the protein synthesis apparatus of the pathogens involved. Thomas Jones is characterising an unusual aminoacyl-tRNA synthetase in the human intracellular parasite *Mycoplasma penetrans*. This parasite presents remarkable genome reduction. However, some of the components of its protein synthesis machinery display new domains of unknown function. We have shown that these modified enzymes are better discriminating catalysts than their *E. coli* homologues. We are now examining how this improvement in substrate recognition is achieved (Jones, Alexander and Ribas de Pouplana, in preparation).

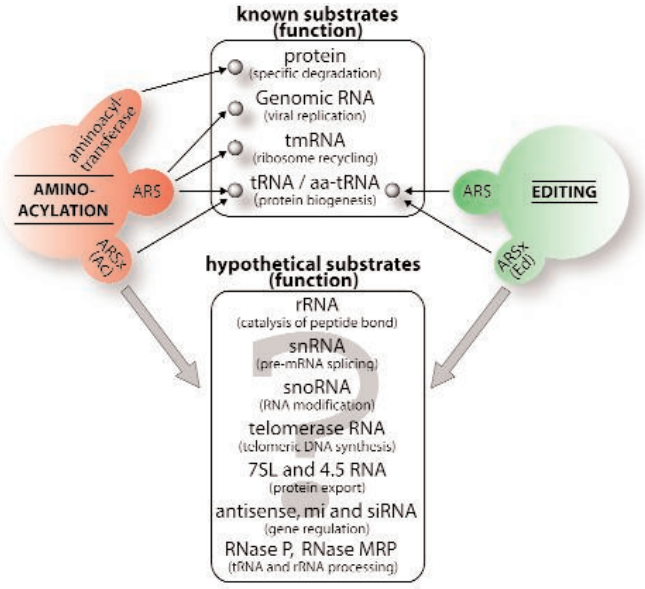

Figure 1. Proposed links between the RNA aminoacylation and deacylation reactions and several cellular pathways unrelated to protein synthesis (Geslain and Ribas de Pouplana, 2004).

Our research also addresses the genome dynamics and the functional role of a family of inflammation-activator domains that are transferred between different protein synthesis enzymes through a ubiquitous process of domain shuffling that is not understood. In the human pathogen *Entamoeba histolytica*, one of these domains doubles its genomic dose through its selective integration in two unrelated enzymes. Manuel Castro is testing the hypothesis that this domain is involved in the induction of local inflammation in the human gut. We are also studying the mechanisms of domain integration involved in this process, and the effect of the newly incorporated domain in the activity of its receiving enzyme (Figure 1).

Finally, the incorporation of Alfred Cortes (ICREA Jr) to the group will allow us to start a new line of research into the mechanisms of gene translation regulation in *Plasmodium falciparum*. This parasite is the main causal agent of malaria, and is responsible for over a million deaths a year. Using a multigenic family of proteins that display differential expression in clonal lines of this parasite, we will examine the

role of chromatin structure and modification in the control of gene expression in *Plasmodium*.

Organelle protein synthesis

We are currently studying the cellular mechanisms that control and coordinate protein synthesis in the cytoplasm and the lumen of cellular organelles, like mitochondria. Most of the proteins required for protein synthesis in the mitochondria are nuclear-encoded, and need to be imported to 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. What prevents the eukaryotic cell from reducing the complexity of its protein synthesis apparatus and from using the same aminoacyl-tRNA synthetases (ARS) in the nucleus and the mitochondria?

To answer these questions we are now examining tRNA aminoacylation in two extreme cases: *Trypanosoma brucei* lysyl-tRNA synthetase (TbKRS) and *Drosophila melanogaster* seryl-tRNA synthetase (DmSRS). *Trypanosoma* are an exception among eukaryotes because their mitochondrial genome does not contain tRNAs. All the tRNAs used in mitochondrial gene expression in *Trypanosoma* are imported from the nucleus. However, some of these tRNAs are still aminoacylated by mitochondria-specific ARS, which also need to be imported. Yaiza Español is currently characterising the mitochondrial TbKRS and comparing it with its cytoplasmic counterpart. Our results indicate that specific mitochondrial modifications may contribute to the maintenance of this complex mechanism. Specific mitochondrial modifications could be related to characteristics of the mitochondrial ribosome and translational apparatus or be indirectly linked to more general nucleic acid modification strategies that occur in *Trypanosoma* organelles.

An equally fascinating case is that of *Drosophila melanogaster*, whose genome apparently codes for two forms of mitochondrial SRS, whose temporal expression seems to be mutually exclusive. Tanit Guitart is currently investigating these enzymes and the reasons behind their unusual distribution.

New methods for the discovery of protein synthesis inhibitors

Major research effort is devoted to the search for improved methods of antibiotic discovery. To this end we are characterising aminoacyl-tRNA synthetases from pathogenic organisms and developing new cellular tools for their expression in human cells (see Geslain *et al*, 2006; Figure 2). Renaud Geslain is studying the mechanisms of recognition that control the correct aminoacylation of $tRNA^{Ser}$ in *Trypanosoma*. Using genetic screens in yeast, he is also developing new tools for the manipulation of ARS structures and the modulation of their substrate specificities. Complementarily, Teresa Bori-Sanz is developing new human cell lines that will express modified ARSs from pathogenic organisms. Our final goal is to develop a new screening procedure for molecular inhibitors of these enzymes.

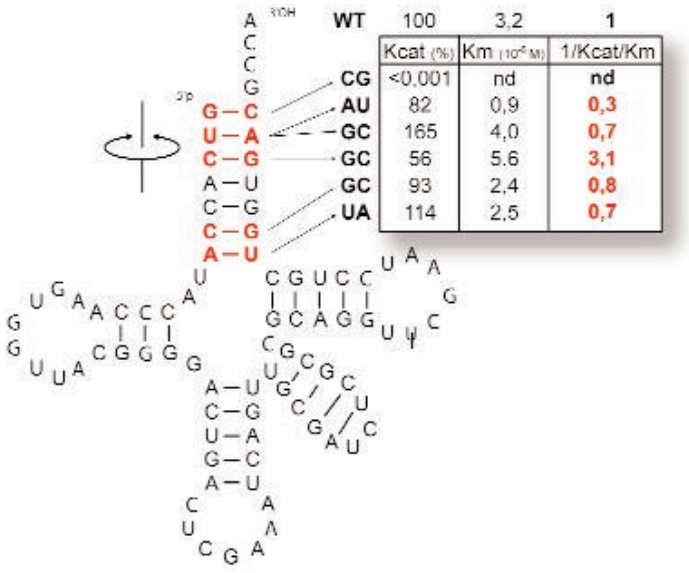

Figure 2. *The characterisation of the recognition of tRNASer by Trypanosoma seryl-tRNA synthetase (Geslain* et al, *2006).*

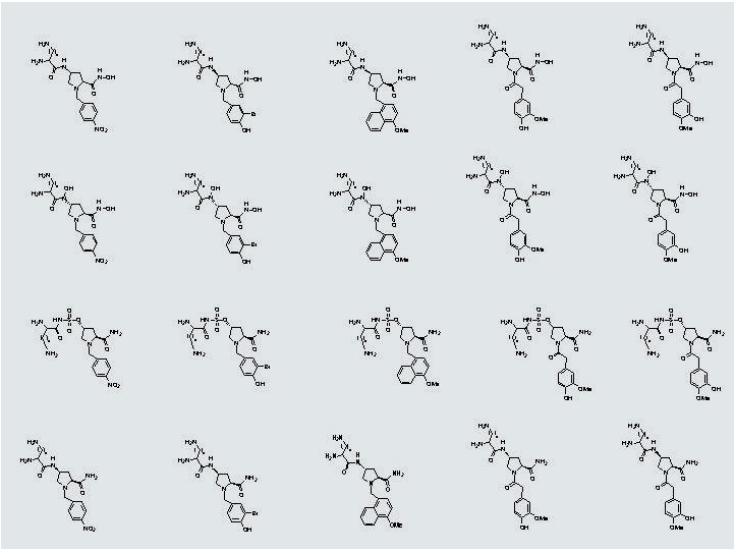

Figure 3. *Some components of a combinatorial library based on analogues of ARS reaction intermediates (Farrera* et al, *in preparation).*

This project is done in collaboration with the Combinatorial Chemistry Unit 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 (Farrera *et al*, in preparation; see Figure 3).

PUBLICATIONS

Benet A, Khong TY, Ura A, Samen R, Lorry K, Mellombo M, Tavul L, Baea K, Rogerson SJ and Cortés A (2006) Placental malaria in women with South-East Asian ovalocytosis. Am J Trop Med Hyg, 75:597-604

Bori-Sanz T, Guitart-Rodés T and Ribas de Pouplana L (2006) Aminoacyl-tRNA synthetases: a complex system beyond protein synthesis. Contributions to Science, in press

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Geslain R, Aeby E, Guitart T, Jones TE, Castro de Moura M, Charrière F, Schneider A and Ribas de Pouplana L (2006) *Trypanosoma* seryl-tRNA synthetase is a metazoan-like enzyme with high affinity for tRNASec. J Biol Chem, 281:38217-38225

Kaestli M, Cockburn IA, Cortés A, Baea K, Rowe JA and Beck H-P (2006) Virulence of malaria is associated with differential expression of *Plasmodium falciparum* var gene subgroups in a case-control study. J Infect Dis, 193:1567-1574

Ribas de Pouplana L (2005) Why does the code include only 20 amino acids? IUBMB Life, 57:523-524

RESEARCH NETWORKS AND GRANTS

Desarrollo de un nuevo método para la selección de antibióticos
Spanish Ministry of Science and Technology grant BIO2003-02611: 2004-2006
Project Coordinator: Lluís Ribas de Pouplana

Discovery of new antibiotics via methods of positive selection
EU Marie Curie International Reintegration Award: 2005-2006
Project Coordinator: Lluís Ribas de Pouplana

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Project Coordinator: Lluís Ribas de Pouplana

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Desarrollo de un nuevo método para la selección de antibióticos
Spanish Ministry of Science and Education grant BIO2006-01558: 2007-2009
Project Coordinator: Lluís Ribas de Pouplana

COLLABORATIONS

Selenocysteine incorporation and organelle protein synthesis in Trypanosoma
André Schneider (Department of Cell and Developmental Biology, University of Fribourg, Switzerland)

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

Codon ambiguity and pathogenicity of Candida albicans
Manuel Santos (Department of Biology, University of Aveiro, Portugal)

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

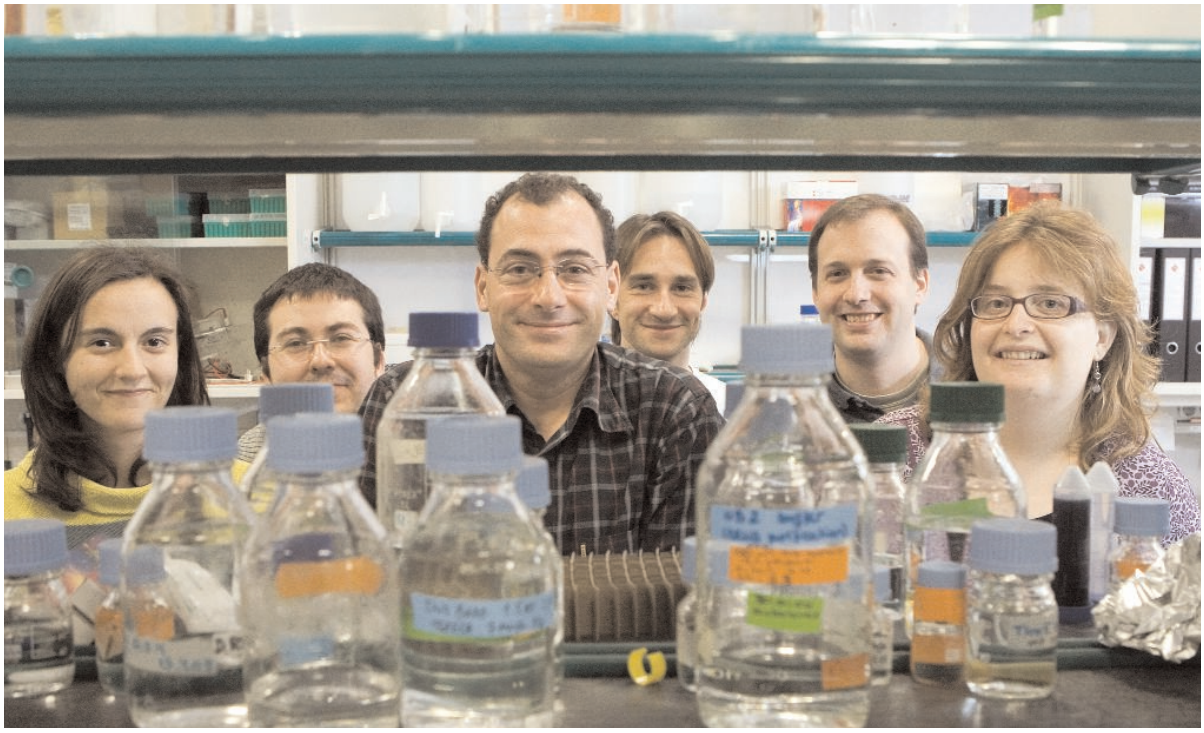
Combinatorial libraries of aminoacyl-adenylate analogues
Miriam Royo (Combinatorial Chemistry Unit, Barcelona Science Park, Spain)

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

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

Expression strategies for Plasmodium *proteins*
ERA Plantech SL, Spain

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



Lluís Ribas de Pouplana's group, March 2006.