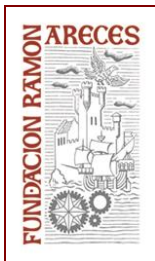


**Simposio Internacional: Biotecnología contra el hambre**  
***International Symposium: Biotechnology to fight hunger***

Madrid, 27 y 28 de octubre de 2011  
*Madrid, October 27-28, 2011*

1. [Nuevos factores implicados en la regulación OXPHOS, Nils Göran-Larsson](#)
2. [PrimPol: un nuevo actor en el metabolismo del mtDNA, Luis Blanco](#)
3. [Nuevos descubrimientos en déficits de complejo III, Cristina Ugalde](#)
4. [Utilización de nucleasas mitocondriales específicas para modificar el nivel de heteroplasmia del mtDNA, Carlos Moraes](#)
5. [Drosophila melanogaster como organismo modelo de patología mitocondrial, Juan Arredondo](#)
6. [¿Es el cáncer una enfermedad mitocondrial?, José Manuel Cuezva](#)
7. [Nuevas perspectivas en la fisiopatología de la enfermedad de Parkinson, Alex Whitworth](#)
8. [Nuevas terapias para MNGIE y otros síndromes de depleción del mtDNA, Ramón Martí](#)
9. [Aproximación a terapia génica de línea germinal de las enfermedades mitocondriales, Shoukhrat Mitalipov](#)
10. [Presente y futuro de la medicina mitocondrial, Massimo Zeviani](#)



**Simposio Internacional: Biotecnología contra el hambre**  
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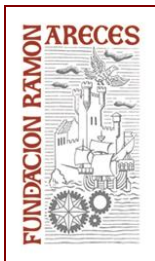
**Nuevos factores implicados en la regulación OXPHOS, Nils Göran-Larsson**

Expression of mtDNA is essential for biogenesis of the oxidative phosphorylation system. It is estimated that ~200 nuclear genes are needed to express the 13 proteins encoded by mammalian mtDNA. These factors include proteins needed for mtDNA maintenance, mtDNA replication, mtDNA transcription, mitochondrial translation and assembly of the respiratory chain complexes. The mtDNA is coated with mitochondrial transcription factor A (TFAM) and packaged into nucleoids of ~100nm in diameter often containing a single copy of mtDNA. TFAM is not only the main protein component of nucleoids but it is also essential for mitochondrial transcription initiation. The basal transcription initiation machinery consists of three components, i.e. mitochondrial RNA polymerase (POLRMT), mitochondrial transcription factor B2 (TFB2M) and TFAM. It is currently debated whether the basal transcription initiation machinery is regulated by additional factors. The mitochondrial transcription termination family of factors consists of four members in mammals, MTERF1-4, that have widely varying roles in regulation of mitochondrial transcription and translation. The recent years has provided and increased insight into regulation of mtDNA expression and many novel factors have been discovered. Key questions still remain to be answered. It is at present unclear if the nucleoid exists in different functional states, depending on the rate of cell division and bioenergetic demands? How is mtDNA transcription regulated? How is mtDNA transcription and translation coordinated?

[VOLVER](#)

**PrimPol: un nuevo actor en el metabolismo del mtDNA, Luis Blanco**  
We describe here a second primase in human cells which has the fascinating ability to start DNA chains with dNTPs, unlike regular primases that use exclusively NTPs. This selectivity for priming with dNTPs (a hallmark of archeal primases) provides this human enzyme with the ability to synthesize *de novo* relatively long DNA chains (up to 4 kb); hence, it is a new DNA polymerase, as well as a primase, and we have accordingly named it PrimPol. By using PrimPol antibodies we demonstrated that about 35% of PrimPol is located inside mitochondria. Accordingly, we demonstrated PrimPol-like activity within human mitochondrial fractions. The same activity was detected in murine mitochondria prepared from wt MEFs, but not from PrimPol-KO MEFs.

PrimPol has a limited insertion fidelity, lacks proofreading, and displays a distributive pattern of elongation. Remarkably, PrimPol is able to polymerize beyond some of the most common oxidative lesions affecting mtDNA, as abasic sites and 8oxoG lesions, and even pyrimidine dimers. Such a combination of primase and polymerase activity, together with its high capacity to tolerate oxidative damage in DNA allows us to propose that PrimPol can facilitate replication fork progression by acting as a translesion polymerase,



**Simposio Internacional: Biotecnología contra el hambre**  
***International Symposium: Biotechnology to fight hunger***

Madrid, 27 y 28 de octubre de 2011  
*Madrid, October 27-28, 2011*

or as a specific primase capable to reinitiate at/beyond lesions that pause or block DNA elongation by Poly. PRIMPOL gene silencing in Hela cells inhibited mtDNA synthesis, affecting mtDNA copy number and mtDNA recovery after EtBr treatment. Primary fibroblasts derived from the murine PrimPol-deficient model showed an altered respiration capacity in the presence of galactose, suggesting a defective maintenance and expression of mtDNA in the absence of PrimPol.

PrimPol probably represents one of the most antique solutions to facilitate replication of small genomes. A “self-sufficient” DNA polymerase, with the capacity to restart DNA synthesis when needed, i.e. to bypass any kind of damage encountered in the template, was probably the best solution before other more sophisticated mechanisms, and a large repertoire of replication and repair DNA polymerases, co-evolved. It is also likely that a PrimPol-like ancestor was probably crucial for the most primitive eukaryotic cells, as they become aerobic, being necessary to cope and tolerate the oxidative damage affecting mitochondrial DNA. Thus, from now on, Poly is not the sole candidate responsible for any kind of DNA synthesis reaction affecting our second genome, thus opening an important avenue to understand the impact of PrimPol in oxidative phosphorylation, mitochondrial function, respiratory stress, and perhaps aging and other mitochondrial-related human pathologies.

[VOLVER](#)

### Nuevos descubrimientos en déficits de complejo III, Cristina Ugalde

Mitochondrial respiratory chain complex III enzyme deficiency is associated with a heterogeneous group of neuromuscular and multi-systemic disorders of variable severity that are present in childhood and adulthood. Despite being considered a relatively uncommon defect of the OXPHOS system, interest has recently shifted towards the Mendelian inheritance of mitochondrial complex III-related disease due to the increasing number of nuclear genetic defects that affect its biogenesis and function. From a clinic-genetic point of view, inherited complex III enzyme deficiency can be classified into disorders due to mutations in complex III structural constituents, or those caused by mutations in assembly chaperones, such as BCS1L and TTC19. The pathophysiological consequences of complex III deficiency-associated mutations were studied in primary skin fibroblasts from six patients harbouring mutations in the *BCS1L* gene. Cells from patients with the most severe clinical phenotypes exhibited slow growth rates in glucose medium, variable combined enzyme deficiencies and assembly defects of the respiratory chain complexes, increased H<sub>2</sub>O<sub>2</sub> levels, unbalanced expression of the cellular antioxidant defences, and apoptotic cell death induction. Besides, all patients showed cytosolic accumulation of the BCS1L protein, suggestive of an impaired mitochondrial import, assembly or stability defects of the BCS1L complex, and fragmentations of the mitochondrial networks that were independent of the respiratory chain dysfunction and ROS levels. The cellular pathways and metabolic adaptations that take place in these

complex III-deficient fibroblasts were next analyzed by two-dimensional DIGE and MALDI-TOF/TOF mass spectrometry, which unambiguously identified 35 protein species significantly altered in the mutant cells. An extensive statistical and cluster analysis of the data revealed a protein profile characteristic for the *BCS1L* mutant fibroblasts that included alterations in energy metabolism, cytoskeleton formation and maintenance, regulation of cell signalling and gene expression, and alterations in the cellular stress response. Our results indicate a shift to anaerobic glycolysis as an adaptive response to mitochondrial dysfunction-elicited oxidative stress, and suggest a metabolic reprogramming that could contribute to explain the clinical manifestations of complex III deficiency.

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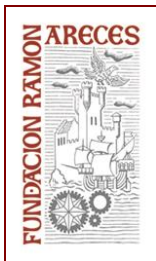
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[VOLVER](#)

#### Utilización de nucleasas mitocondriales específicas para modificar el nivel de heteroplasma del mtDNA, Carlos Moraes

Mitochondrial diseases are commonly caused by high levels of heteroplasmy mutations in the mitochondrial DNA (mtDNA). In an attempt to reduce the mutant mtDNA load, we have used restriction endonucleases that can specifically promote double-strand breaks in the mutant genome. Although we found this approach to be highly efficient, the limitation of the recognition sites of known nucleases narrowed the application of the technology. To expand this approach, we engineered TAL-effector nucleases (TALEN) to localize to mitochondria. We designed and engineered two different mitochondrial-targeted TALENs (mitoTALENs) to bind and cleave two different mtDNA pathogenic mutations: the “common deletion of 5kbp” (associated with ocular myopathies and Kearns-Sayre syndrome) and a G14459A point mutation in the ND6 subunit of Complex I (associated with optic atrophy and dystonia). Cybrid cells harboring heteroplasmic levels of the mutated mtDNAs were transfected with the mitoTALENs and Flow sorted for fluorescent markers after 48hs. Cells transfected with the MitoTALENs were able to reduce the levels of the pathogenic mtDNA common deletion (5kb deletion) and the levels of the point mutation G14,459A in the respective cybrids, in both cases increasing the levels of WT mtDNA. No significant



**Simposio Internacional: Biotecnología contra el hambre**  
**International Symposium: Biotechnology to fight hunger**

Madrid, 27 y 28 de octubre de 2011  
Madrid, October 27-28, 2011

depletion of total mtDNA was found after two weeks. These findings raise the possibility that mitoTALENs can be beneficial to a large subgroup of patients with mitochondrial diseases associated with heteroplasmic mtDNA.

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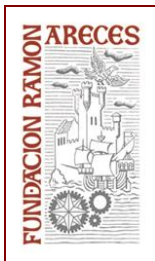
### ***Drosophila melanogaster* como organismo modelo de patología mitocondrial, Juan Arredondo**

Mitochondria are organelles of vital importance in eukaryotes that play a very important role in cell metabolism having a critical role in a great number of processes. Nevertheless, its function is the production of energy in the form of ATP by a process called oxidative phosphorylation (OXPHOS). Mitochondrial disorders (MIDs), due to mutations in either nuclear or mitochondrial DNA, most often cause a malfunction of the respiratory chain (RC) and therefore, a defective OXPHOS function. Respiratory chain dysfunction is the most frequent in born error of metabolism with an incidence of 1/5000 live birth. Defects in cytochrome c oxidase (COX) or complex IV, due to mutations in COX assembly factors Sco2 or Surf1, are one of the most common in the OXPHOS system. These patients present a wide combination of clinical symptoms, from Leigh Syndrome and other rare neuromuscular syndromes to cardiomyopathy to mention some of them. Moreover, is quite common that since neuro-muscular symptoms are very strong, defects in other tissues, as the heart, are often overseen.

*Drosophila melanogaster* is an invertebrate genetic model system that has emerged as a powerful tool for analyzing heart development and differentiation processes. In the recent years, *Drosophila* has emerged as a powerful model system for heart human disease, allowing, in some cases, to identify new genes involved in human cardiomyopathies. Using *Drosophila melanogaster* as model system, we have set up the heart specific interference of ScoX (the homolog of the human Sco1 & 2 factors) using a UAS-GAL4 driven RNAi strategy to examine its role *in vivo* in cardiac physiology. Analysis of cardiac physiology is carried out using high-speed video recording of beating hearts in conjunction with movement analysis algorithms. We have measured the RNA interference level by qPCR using Taq-man probes and its structural consequences in the *Drosophila* heart by immunofluorescence and confocal microscopy.

We have measured the relative RNA levels from isolated hearts of control and tin ScoX flies. Interfered flies display a 50% reduction in ScoX expression. Heart physiology analysis in adult animals showed a cardiac dysfunction evidenced in a reduced fractional shortening (FS) due to a bigger systolic diameter, an increased incidence of long diastoles and prolonged diastolic and systolic intervals. Consistent with the data obtained from live beating hearts, cardiac dilation was also seen in fixed tissue. Structural analysis a large increase in conical chamber size in both ScoX interfered flies. Adult hearts showed abnormalities in the cellular architecture of cardiomyocytes, being specially apparent the presence of severely misaligned myofibrils as well as some gaps or holes. In this context we have also analysed the metabolic state of the fly heart, finding a glycolytic shift evidenced by a transcriptional upregulation of all analyzed genes. No changes have been





**Simposio Internacional: Biotecnología contra el hambre**  
***International Symposium: Biotechnology to fight hunger***

Madrid, 27 y 28 de octubre de 2011  
*Madrid, October 27-28, 2011*

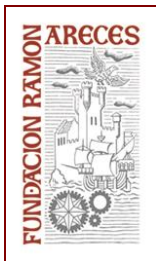
observed in nuclear encoded OXPHOS genes in contrast to mitochondrial transcripts that display a strong decrease in its expression levels. The observed phenotypes are reminiscent of a dilated cardiomyopathy and correlate well with the described phenotypes in human patients, confirming the notion that *Drosophila* might be a suitable model to study mitochondrial diseases.

[VOLVER](#)

¿Es el cáncer una enfermedad mitocondrial?, José Manuel Cuezva  
No, it is not. Cancer is a complex disease in which abnormal cells divide without control and are able to invade other tissues. However, a most relevant phenotypic trait of cancer cells is the reprogramming of cellular metabolism to satisfy the demand of carbon skeletons and energy that is required for the biosynthesis of macromolecules in the daughter cells. Here is one aspect in which mitochondria come into picture because they are essential organelles in promoting rewiring of metabolism during proliferation. Indeed, many of the metabolic pathways that reside in mitochondria such as decarboxylation of pyruvate, the TCA cycle,  $\beta$ -oxidation of fatty acids and oxidative phosphorylation are very much diminished during proliferation whereas the activity of glycolysis and of the pentose phosphate pathway are profoundly enhanced. An additional aspect in which mitochondria are also critical organelles is in deciding and executing cell death, a trait that is also altered in cancer cells and that is molecularly and functionally integrated with its bioenergetic function. A critical hub in the reprogramming of energy metabolism and cell fate is the mitochondrial  $H^+$ -ATP synthase, the engine that synthesizes most of the ATP that sustains cellular activity of the normal differentiated cell and that also forms part of the molecular machinery that is required to execute apoptosis.

Two main mechanisms are used by human carcinomas of the breast, colon and lung to silence the  $H^+$ -ATP synthase, hence contributing to metabolic reprogramming to an enhanced aerobic glycolysis and for promoting a cell-death resistant phenotype. One is the translation masking of the mRNA that encodes the catalytic subunit of the  $H^+$ -ATP synthase ( $\beta$ -F1-ATPase) which is mediated by RNA binding proteins that upon binding onto the mRNA impede its efficient translation. A second one involves the over-expression of the ATPase Inhibitory Factor 1 (IF1), a short-lived protein that by inhibiting the  $H^+$ -ATP synthase not only contributes to metabolic rewiring but also retrograde signals to the nucleus of the cancer cell features of the oncogenic phenotype. In this presentation we will summarize these findings to further emphasize the tumor suppressor role of mitochondrial oxidative phosphorylation, a promising new therapeutic target of cancer.

[VOLVER](#)



**Simposio Internacional: Biotecnología contra el hambre**  
**International Symposium: Biotechnology to fight hunger**

Madrid, 27 y 28 de octubre de 2011  
Madrid, October 27-28, 2011

## Nuevas perspectivas en la fisiopatología de la enfermedad de Parkinson, Alex Whitworth

Genetic analysis of Parkinson's disease (PD) has identified single-gene mutations responsible for rare, heritable forms of PD have been identified. Understanding the consequence of these mutations can potentially illuminate the pathogenic mechanisms underlying many forms of PD. These mutations can be modelled in genetically tractable model systems, such as the fruit fly, *Drosophila melanogaster*, which has proven a remarkably informative model.

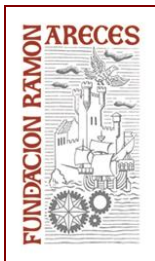
Loss of function in *parkin* and *PINK1* cause the majority of early onset PD. It was established using *Drosophila* that PINK1 and parkin act in a common pathway to maintain mitochondrial homeostasis. Mitochondria-localised PINK1 is required to signal parkin translocation to dysfunctional mitochondria where ubiquitination of multiple outer membrane targets, such as Mitofusins, promotes their isolation and degradation by autophagy (mitophagy). The inability to properly regulate mitochondrial turnover will impact on the long-term maintenance of the mitochondrial network and ultimately on neuronal survival, suggesting a possible pathogenic mechanism for PD, however, the physiological *in vivo* evidence is limited. In addition, many details of the molecular mechanism and other pathway components are unclear. Our work aims to address these deficiencies using *Drosophila* as a model system.

[VOLVER](#)

## Nuevas terapias para MNGIE y otros síndromes de depleción del mtDNA, Ramón Martí

Los síndromes de depleción y deleciones del DNA mitocondrial (MDDS) son enfermedades mitocondriales de herencia autosómica cuya característica definitoria es la presencia de alteraciones cuantitativas (reducción del número de copias, o depleción) y/o cualitativas (deleciones múltiples, mutaciones puntuales) en el DNA mitocondrial (mtDNA) de algunos tejidos de los pacientes. Estas alteraciones no son heredadas de la madre, sino que se producen en el paciente de manera somática a través mecanismos bioquímicos relacionados con la alteración genética específica que los causa.

Entre los genes implicados en este grupo de enfermedades, existe un subgrupo que codifica proteínas relacionadas con el metabolismo de los desoxiribonucleótidos. Cuatro genes forman parte de este grupo de manera inequívoca: *TYMP* (codifica la timidina fosforilasa ó TP, implicada en el catabolismo de la timidina y desoxiuridina), *TK2* (codifica la timidina kinasa 2 ó TK2, kinasa mitocondrial de desoxiribonucleósidos de pirimidina), *DGUOK* (codifica la desoxiguanosina kinasa ó dGK, kinasa mitocondrial de desoxiribonucleósidos de purina) y *RRM2B* (codifica la subunidad pequeña p53-inducible de la ribonucleótido reductasa ó p53R2). En todos estos casos, el mecanismo bioquímico común que interfiere con la replicación del mtDNA es la disponibilidad limitada de uno o varios desoxiribonucleósidos trifosfato (dNTPs) que son los sustratos necesarios para la replicación del DNA. Por tanto, una posible estrategia terapéutica común es el suministro de aquel o aquellos sustratos que se encuentran reducidos en cada caso concreto. Los



**Simposio Internacional: Biotecnología contra el hambre**  
***International Symposium: Biotechnology to fight hunger***

Madrid, 27 y 28 de octubre de 2011  
*Madrid, October 27-28, 2011*

nucleótidos son moléculas cargadas a pH fisiológico, y no pueden atravesar las membranas celulares sin ayuda de transportadores específicos. Ello, y la presencia de fosfatasa en los fluidos biológicos, hace que la biodisponibilidad de estas sustancias sea extremadamente reducida. Una alternativa es el suministro de sus precursores bioquímicos, los desoxiribonucleósidos. Algunos resultados de estudios previos, y trabajos recientes de nuestro grupo demuestran que esta estrategia puede prevenir la depleción del mtDNA en cultivos celulares y en modelos animales. Por tanto, el suministro de desoxiribonucleósidos (y/o la inhibición de su catabolismo) se presenta como una posible estrategia terapéutica.

Por otra parte, el déficit de TP da lugar a un caso especial de MDDS, el MNGIE (encefalomiopatía neurogastrointestinal mitocondrial) que se debe a la acumulación tóxica de los substratos de TP, timidina y desoxiuridina. Para esta enfermedad, cuyo tratamiento en la actualidad consiste en el trasplante alogénico de progenitores hematopoyéticos, hemos desarrollado estudios preclínicos que indican que la terapia génica puede ser una muy buena alternativa en un futuro no muy lejano.

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**Aproximación a terapia génica de línea germinal de las enfermedades mitocondriales, Shoukhrat Mitalipov**

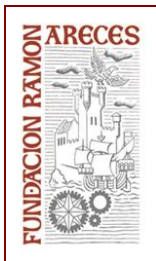
Mitochondria are critical for basic cellular function due to their principal role in the production of energy. Mitochondria contain their own genome (mtDNA) which, unlike the nuclear genome, is exclusively transmitted maternally from the egg. Mutations in mtDNA occur at a 10 to 100-fold rate higher than in nuclear DNA and cells usually contain a mixture of mutant and normal mtDNA, a phenomenon known as heteroplasmy. When the mutant load reaches a certain threshold, mitochondrial function is impaired leading to serious human disorders, including myopathies, neurodegenerative diseases, diabetes, cancer and infertility.

To establish preclinical models for new therapeutic approaches, we initially demonstrated that the mitochondrial genome can be efficiently replaced in mature metaphase II arrested monkey oocytes (Tachibana et al., Nature 2009). The reconstructed oocytes with the mitochondrial replacement were capable of supporting normal fertilization, embryo development and produced healthy offspring.

More recently, we demonstrated the feasibility and efficacy of mitochondrial genome replacement in human oocytes donated by healthy volunteers (Tachibana et al., Nature 2013). To measure success, we fertilized reconstructed oocytes in vitro and assessed the normality of fertilization, embryo development to blastocysts and embryonic stem cells.

These studies suggest that the nuclear genetic material from a patient's egg containing mtDNA mutations could be removed, and transplanted into an enucleated egg containing normal mtDNA donated by a healthy female. A child born following fertilization





**Simposio Internacional: Biotecnología contra el hambre**  
***International Symposium: Biotechnology to fight hunger***

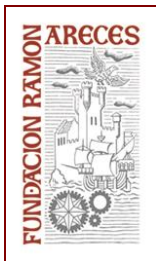
Madrid, 27 y 28 de octubre de 2011  
*Madrid, October 27-28, 2011*

with the husband's sperm would be free of risk from maternal mtDNA mutations as well as the authentic biological child of the patients.

[VOLVER](#)

### Presente y futuro de la medicina mitocondrial, Massimo Zeviani

Mitochondria are the major source of ATP that is synthesized by the respiratory chain (RC) through the process of oxidative phosphorylation (OXPHOS). ATP is the principal energy substrate required for all active processes within the cell, and ATP deficiency leads to cellular dysfunction and ultimately cell death. OXPHOS is a complex biochemical process, carried out through the dual control of physically separated, but functionally interrelated, genomes, nuclear and mitochondrial DNAs. Faulty energy supply due to defective OXPHOS is the biochemical signature of mitochondrial disorders, a group of highly invalidating human conditions, for which no effective treatment is nowadays available. Mitochondrial disorders strike >1 in 5,000 children and adults and predominantly affect the nervous system and skeletal muscle, causing substantial morbidity and premature death. The genetic and biochemical intricacy of mitochondrial bioenergetics explains the extreme heterogeneity of mitochondrial disorders, a formidable challenge for both diagnostic workup and treatment. Research collaborations forged through different clinical, translational and basic research programs have led to major advances in understanding the pathophysiology of these diseases, and to the development of novel cellular and animal models. Nevertheless, it is still not clear how cells become dysfunctional in response to a mitochondrial biochemical defect. Although bioenergetic failure associated with reduced ATP biosynthesis appear to be critical in individual or combined MRC-complex defects, other mechanisms are also likely to be involved, and are probably predominant in the pathogenesis of specific syndromes, such as alterations of cellular redox status, the production of reactive oxygen species, compromised  $\text{Ca}^{2+}$  homeostasis, dysregulation of the proton-motive force and its dissipation, and mitochondrial pathways of apoptosis. The tremendous clinical, biochemical and molecular heterogeneity of mitochondrial disorders makes virtually each member of the whole mitochondrial proteome a candidate for disease. As a result, only 40% of adult-onset disorders are currently diagnosed at the molecular level, and much lesser so in infantile syndromes. However, new technological and biocomputational tools offer the possibility of rapid and affordable analysis of the exome, i.e. the coding regions of all genes in single individuals or small families. Mitochondrial disease proteins can then be selected by exploiting predictive softwares, dedicated databases, and ex vivo experiments. We have identified several new disease genes, each responsible of distinct defects of the respiratory chain, mtDNA metabolism, or both. Structural analysis has allowed us to dissect out the molecular consequences of the ablation or defects of these proteins, and their physical status in normal and disease conditions. To gain further insight on function and mechanism of disease, we have then created specific recombinant lines in yeast, flies, and mice. These



**Simposio Internacional: Biotecnología contra el hambre**  
***International Symposium: Biotechnology to fight hunger***

Madrid, 27 y 28 de octubre de 2011  
*Madrid, October 27-28, 2011*

models have also been exploited to implement experimental therapeutic strategies, based on gene and cell replacement, or pharmacological control of mitochondrial biogenesis.

[VOLVER](#)

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