Genome maintenance and variability: enzymology of DNA replication and repair
Luis Blanco Dávila

Molecular bases of viral and fungal cytopathology
Luis Carrasco Llamas

Post-transcriptional regulation of gene expression in eukaryotes
César de Haro Castella

Functional organization of the mammalian genome
Maria Gómez Vicentefranqueira

Cell division, genome replication and chromatin
Crisanto Gutierrez

Internal initiation of translation in eukaryotic mRNAs
Encarnación Martínez-Salas

Regulation of gene expression in Leishmania
Jose Maria Requena Rolanía

Replication of bacteriophage ø29 DNA African Swine Fever Virus
Margarita Salas Falgueras

Chromosome replication and genome stability
José Antonio Tercero Orduña

Repair of bacterial DNA group
Miguel de Vega José
Genome maintenance and variability: enzymology of DNA replication and repair

Research Summary

Since the last 15 years, our group studies two eukaryotic DNA polymerases (Polλ and Polμ), discovered in Blanco laboratory, that are involved in DNA double-strand break repair (DSBs) by nonhomologous end joining (NHEJ). In the last two years we showed the role of a specific motif (“brooch”) and a network of conserved interactions that facilitate both end-bridging at the DSB, and the adequate positioning of Loop1 during NHEJ by Polμ. We also demonstrated that NTPs are ultimate Polμ substrates during NHEJ, the propensity of Polμ to generate nucleotide expansions on iterative sequences, and its regulation via phosphorylation of BRCT and Loop 1 by Cdk2-cyclinA. We also studied the yeast and bacterial orthologues of these human polymerases, demonstrating a direct role in chromosomal translocations and damage tolerance. In collaboration with Aidan Doherty (GSDC, Univ Sussex, UK) we have completed the characterization of Mycobacterium tuberculosis PolDom, involved in the NHEJ pathway, whose mechanism was shown to be convergent with that of Polλ and Polμ. In vivo analysis of Polλ and Polμ function is being carried out by using cellular and mouse models of deficiency in one or both of these enzymes. In collaboration with Antonio Bernad (CNB, Madrid), Polμ was shown to have an impact in genome instability and aging. Finally, we have characterized a novel primase/polymerase (PrimPol) in human cells, and shown its involvement in damage tolerance and maintenance during replication of both nuclear and mitochondrial DNA, being able to reprime forks stalled as a consequence of replicative stress. These studies, initiated in collaboration with Ian Holt (MRC, UK), and Juan Méndez (CNIO, Spain), will be continued with the analysis of a mouse model of PrimPol deficiency (KO) which is viable, paying special attention at mitochondrial-dependent phenotypes, and at its value as a model for aging and tumorigenesis.
Genome maintenance and variability: enzymology of DNA replication and repair

Figure 1. Selecting the Templating Base: Roles of Residues Phe63 and Phe64. A cartoon showing the dichotomy that PolDom confronts when dealing with gaps longer than 1 nt during NHEJ: the template strand is either “scrunched,” and the gap filled-in correctly (left side), or the template strand is dislocated and sequence is lost with the production of frameshifts (right side). Phenylalanines Phe63 and Phe64 are shown as blue hexagons holding the kink in the DNA substrate (yellow).

Figure 2. Alternative solutions for translesion DNA synthesis by human PrimPol. (A) DNA replication is stalled when the replicative DNA polymerase encounters a lesion in the template DNA, accumulating ssDNA ahead of the lesion. (B) PrimPol is able to resolve this problem by 3 different mechanisms: 1) directly reading lesions as 8oxoG, acting as a conventional TLS polymerase; 2) skipping unreadable lesions (abasic sites, 6-4PP...) by microhomology-mediated primer realignment ahead of the lesion; 3) synthesizing a new primer ahead of the lesion, as a TLS primase. (C) PrimPol can be considered the archetype of more conventional primases operating in the lagging strand, that are specialized in making RNA primers exclusively.
Genome maintenance and variability: enzymology of DNA replication and repair

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Fabiana dos Santos Rando

**R&D Project Manager:**
Estefanía Martínez Jover
Genome maintenance and variability: enzymology of DNA replication and repair

Publications (1)


Genome maintenance and variability: enzymology of DNA replication and repair

Publications (2)


Genome maintenance and variability: enzymology of DNA replication and repair

Other Activities

- Organizer of the Cantoblanco Workshop: “Polymerases involved in DNA replication, repair and mutagenesis”, held at CBMSO (Madrid), in June 2012.
- Best Patent Award from the madri+d Foundation: "Chimaera of ø29 DNA polymerase".
- 2012/2013 Award to the Best PhD thesis of the CBMSO, to Sara García-Gómez.
- Carmen and Severo Ochoa Research Award in Molecular Biology (2014), to Luis Blanco.
Genome maintenance and variability: enzymology of DNA replication and repair

Patents

Genome maintenance and variability: enzymology of DNA replication and repair

Doctoral Theses


**Ana Gómez Bedoya (2013).** Análisis estructura-función de la DNA polimerasa lambda humana y su implicación en la reparación del DNA mediante NHEJ. Universidad Autónoma de Madrid. Supervisor: Luis Blanco Dávila.

Molecular bases of viral and fungal cytopathology

Research Summary

The mechanisms that regulate translation of cellular and viral mRNAs have been analysed, as well as the cytopathogenic effects of individual viral proteins on mammalian cells. In recent past, we focussed our interest in two types of viral proteins: proteases and viroporins. In addition, we have devoted some efforts to elucidate the presence of fungal infections as the potential etiology of several neurodegenerative diseases, including multiple sclerosis and Alzheimer’s disease.

Translational regulation of cellular and viral mRNAs. The action of viral proteases on the translation of different mRNAs has been analysed. We have also studied the redistribution of cellular proteins between the nucleus and the cytoplasm. Notably, the picornavirus 2Apro and Lpro are able to provide eIF2 independence for the translation of viral mRNAs. Some viral mRNAs can be translated by a dual mechanism and require different initiation factors depending on the translation context. In this regard, Sindbis virus constitutes a good model system for these studies. Translation of the subgenomic mRNA from this virus does not require several initiation factors, such as eIF4A in the infected cells (Figure 1). However, eIF4A is necessary for translation for this mRNA in in vitro systems. At present, several constructs bearing different structures in the subgenomic mRNA have been obtained, in order to determine the precise mechanism of its initiation.

Neurodegenerative diseases. A number of assays have been developed to detect the presence of fungal infections in peripheral blood, cerebrospinal fluid and brain samples from patients diagnosed with multiple sclerosis or Alzheimer’s disease (Figure 2). Elevated levels of fungal macromolecules have been detected, such as polysaccharides, proteins and DNA. These findings open a new field of research on the etiology of these neurodegenerative diseases.
Molecular bases of viral and fungal cytopathology

**Figura 1.** Effect of inhibitors of translation initiation factors eIF2, tapisargin (Tg) or sodium arsenite (Ars) and eIF4A, hippuristanol (hipp) on protein synthesis from mock-infected cells and from cells infected with Sindbis virus in mouse or insect cells.

**Figura 2.** Immunohistochemistry analysis of fungal infection in human frontal cortex from an Alzheimer’s disease patient. DAPI staining (blue), fungal cells (green) and neurofilaments (red).
Molecular bases of viral and fungal cytopathology

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Diana Pisa García
Molecular bases of viral and fungal cytopathology

Publications


Post-transcriptional regulation of gene expression in eukaryotes

Research Summary

In response to different environmental stresses, including viral infection, nutrient deprivation, and ultraviolet light exposure, the transient phosphorylation of the α subunit of translation initiation factor 2 (eIF2α) rapidly reduces global protein synthesis, which lowers energy expenditure and facilitates reprogramming of gene expression to remediate stress damage. Our recent work has been focused on these major lines:

1) Regulation of cell cycle and sexual differentiation by eIF2α kinases in Schizosaccharomyces pombe. There is a differential response of the three members of this kinase family to nutrient deprivation-mediated stress, being phosphorylation of eIF2α essential for the proper G1-phase cell cycle arrest and for the cell mating in the absence of nitrogen, leading to their survival.

2) Ccr4-Not complex is a coordinator of different aspects of gene expression regulation, from mRNA synthesis to degradation. We have studied the relationship of several Ccr4-Not complex proteins with stress response in Schizosaccharomyces pombe. Through protein-protein interaction and genetic relationship we have unravel the mechanistic links between stress-activated MAPKs and Ccr4-Not complex.

Recently, our group and Dr Iván Ventoso group have joined together in order to better develop our objectives: i) to study the mechanisms by which cells detect and respond to distinct stress forms (ultraviolet radiation, nutrient deprivation) through the activity of eIF2α kinases; ii) exhaustive description of the translational reprogramming in response to stress, by using mouse and fission yeast S. pombe as models; iii) identification of mRNA sequences and structures, together with protein factors, involved in the above mentioned reprogramming of gene expression; iv) to study the implications of these processes in longevity and in the development of age-related diseases (cancer).
Post-transcriptional regulation of gene expression in eukaryotes

Figure 1. In S. pombe eIF2α phosphorylation is required for proper G1 phase cell cycle arrest when growing in the absence of nitrogen. (A) Using flow cytometry, it is observed that in the absence of nitrogen eIF2αS52A cells, which express a non-phosphorylatable eIF2α, significantly retard arrest in G1 phase of the cell cycle. (B) The optical (Nomarski) and fluorescent (DAPI) micrographs show that, unlike the wild-type cells (WT) which move from G2-M to G1 by division and rounding, eIF2αS52A cells continue to elongate without producing cell division.

Figure 2. Scheme of translation reprogramming during stress response in mouse and fission yeast.
Post-transcriptional regulation of gene expression in eukaryotes

Group Leader:
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Post-transcriptional regulation of gene expression in eukaryotes

Publications


Post-transcriptional regulation of gene expression in eukaryotes

Other Activities

- Organización de actividades I + D
Post-transcriptional regulation of gene expression in eukaryotes

Doctoral Theses

Marina Portantier (2013). Papel del complejo Ccr4-Not en la respuesta a estrés mediada por la MAPK Spc1 en Schizosaccharomyces pombe. Universidad Autónoma de Madrid. Director: Miguel Ángel Rodríguez Gabriel.
Functional organization of the mammalian genome

Research Summary

The long-term interest of our laboratory is decoding the regulatory information of the mammalian genome, focusing on the coordination between transcription and replication that is needed to respond to either environmental changes or determinants of cell identity. Within this context, we combine detailed molecular biology approaches directed to characterise the sites of DNA replication initiation and their epigenetic requirements, with genome-wide approaches aimed to reveal the complexity of the organization of both basic genomic processes. As model systems we use human and mouse cells, both wild-type and mutant for several key chromatin components and regulators.

During the last years several efforts to map replication origins genome-wide in mammalian cells revealed that, although DNA synthesis can start at multiple genomic sites, those co-localizing with gene promoters are more efficiently activated and more conserved across cell types. We have recently demonstrated that replication initiation sites at these efficient promoter-origins occur at positions of high nucleosome occupancy. The binding sites for the Origin Recognition Complex (ORC), however, occur at adjacent but distinct positions marked by labile nucleosomes. Derived from these studies, our working hypothesis is that nucleosome architecture at replication origins dictates the start sites of leading strand synthesis and that the reposition of these specific nucleosomes behind the replication fork could provide the opportunity to change the chromatin structure that could promote a switch during cell differentiation and development. Our studies aim to provide fundamentally new and important insight into the mechanisms by which transmitting genetic and epigenetic information of the regulatory regions that control replication and transcription initiation sites are coupled. This knowledge will likely contribute to a better understanding of the complex role of chromatin in regulating the genome versatility and stability. In addition, understanding the molecular interactions between ORC and nucleosomes might have implications for interfering with this type of ORC-origin binding to control cell division.
Functional organization of the mammalian genome

Figure 1. Distribution of ORC binding sites and labile nucleosomes at efficient replication origins in human cells.

Figure 2. Nucleosome dynamics at the human replication origin LaminB2 upon S-phase entry.
Functional organization of the mammalian genome

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Functional organization of the mammalian genome

Publications


Functional organization of the mammalian genome

Other Activities

- Scientific co-organizer of the biannual Madrid Chromatin Club Meetings, CBMSO.
Cell division, genome replication and chromatin

Research Summary

The transition to multicellularity required the evolution of novel structures and mechanisms to coordinate cell division, acquisition of cell fates and the differentiation, and establishment of complex regulatory networks. Our group is interested in understanding the mechanisms that control these processes and how epigenetic mechanisms affect such coordination.

To that end, we use the model plant *Arabidopsis thaliana* that offer us the possibility of carrying out molecular, cellular, genetic and genomic approaches. In addition, plant development, contrary to the situation in animals, is post-embryonic and occurs during the entire life of the organism. Our research is aimed at understanding fundamental questions on cell proliferation control, cellular homeostasis and genome replication in multicellular organisms.

Cell proliferation is crucial for organogenesis, which is determined by a strict control of gene expression patterns.

We study chromatin dynamics along the cell cycle with special emphasis in two aspects: one, the regulation of cell proliferation potential, very related to the control of gene expression in G1 and G2, and the exit to differentiation, and another, related to genome replication. This implies that not only DNA but chromatin needs to be duplicated every cell cycle. In turn, several stages of genome replication are associated with specific chromatin states. We have identified 9 distinct chromatin states that define the Arabidopsis genome based on specific combinations of epigenetic marks (signatures). We are also developing genomic strategies to study the functional properties of replication origins in all cell types of the whole organism to determine the influence of hormonal conditions, developmental signals and the environment. This approach is allowing us to use mutants to study genome replication.
Cell division, genome replication and chromatin

Figure 1. The Arabidopsis genome (Chr 1) in 9 color-coded chromatin states.

Figure 2. Identification of histone H3.1 and H3.3 in the Arabidopsis root.
Cell division, genome replication and chromatin

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Cell division, genome replication and chromatin

Publications


Cell division, genome replication and chromatin

Other Activities

- Editorial Board EMBO J., EMBO Rep. Editor Plant J.
Cell division, genome replication and chromatin

Doctoral Theses

Nuria Mauri Panadero (2013) GEM, una proteína don dominio GRAM, es un regulador negativo de la señalización por ABA durante la germinación en *Arabidopsis thaliana*. Universidad Autónoma de Madrid. Director: Crisanto Gutiérrez.
Internal initiation of translation in eukaryotic mRNAs

Research Summary

Our main goal is to understand alternative mechanisms of translation initiation in eukaryotes. Internal Ribosome Entry Site (IRES) elements are mRNA regions that govern internal initiation of translation. To achieve its function, IRES elements assemble ribonucleoprotein complexes in which RNA structure and IRES function is tightly coupled. During this period we have characterized the function of Gemin5 as a negative regulator of IRES-dependent translation. Gemin5 binds to a discrete IRES stem-loop through its C-terminal region. Binding of Gemin5 allows a large degree of sequence flexibility and requires a short hairpin surrounded by C or U-rich sequences. Interaction of Gemin5 with the IRES competes out the binding of PTB, a protein stimulating IRES activity, explaining at least in part its negative effect on translation initiation. Analysis of the protein domains involved in IRES recognition revealed the presence of a bipartite non-conventional RNA-binding motif, where RBS1 binds RNA with higher affinity and RBS2 harbors the translation control motif (Fig 1).

Evolutionary conserved RNA motifs determine its tertiary structure. Knowledge of the structural organization of a conserved essential subdomain allowed the prediction of genome sequences potentially adopting IRES-like structural motifs. Using Inverse Folding, we have found that a short sequence within the open-reading-frame of *Drosophila melanogaster* TAF6 can confer weak but positive translation initiation, reinforcing the idea that this is a useful tool to predict IRES-like structural motifs. In addition, we have shown recently that IRES folding depends on divalent ions concentration (Fig. 2). This study showed that RNA structure is flexible at concentration of divalent ions close to physiological concentrations and allows its recognition by eIF4G, a factor needed for IRES activity. In contrast, high Mg²⁺ concentration induces a constrained RNA structure, precluding the binding of this factor, and explaining the lack of IRES activity at high salt conditions.
Internal initiation of translation in eukaryotic mRNAs

Figure 1. RNA-binding site of Gemin5

Figure 2. SHAPE structural analysis of IRES elements
Internal initiation of translation in eukaryotic mRNAs

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Internal initiation of translation in eukaryotic mRNAs

Publications


Internal initiation of translation in eukaryotic mRNAs

Other Activities

• E Martinez-Salas is Member of the Editorial Board of Virology
Regulation of gene expression in *Leishmania*

Research Summary

One of the most remarkable features of *Leishmania* is the almost complete absence of transcriptional regulation. In these organisms, gene expression is regulated by post-transcriptional mechanisms poorly understood at present. Therefore, these organisms are extremely attractive models for studying mechanisms of post-transcriptional regulation. In addition, these unicellular parasites are important pathogens for humans.

The main interest of our laboratory is contributing to the knowledge of mechanisms by which gene expression in *Leishmania* is controlled. We are working in the identification of both regulatory cis-elements, often found in the 3' untranslated regions (UTRs) of mRNAs, and RNA-binding proteins (RBPs), as key players in the regulation of gene expression. However, a constraint for these studies was the deficient annotation existing in *Leishmania* genome databases, genes lack annotated 5'- and 3'-UTRs. Recent advances in sequencing technology have created unprecedented opportunity to define transcriptomes. For this purpose, in collaboration with the Genomics and Massive Sequencing Unit at CBMSO, headed by Dr. Begoña Aguado, we have reconstructed the entire transcriptome of *Leishmania major* and other Leishmania species using deep RNA sequencing (RNA-Seq). Such information is central to determining the timing and regulation of gene expression in different developmental stages and the identification of functional elements in mRNAs.

On the other hand, as members of the Tropical Diseases network (ISCIII; [http://www.ricet.es/es/](http://www.ricet.es/es/)), our group is engaged in collaborative projects exploring new therapeutic and preventive strategies to control leishmaniasis, a disease that continues affecting millions of people worldwide. Also, we are participating in a project granted by the European Commission’s FP7 Cooperation Work Program for Health; this project entitled Clinical Studies on a Multivalent Vaccine for Human Visceral Leishmaniasis (MuLeVaClin) is aimed to develop a vaccine against human visceral leishmaniasis ([http://www.mulevaclin.eu](http://www.mulevaclin.eu)). Finally, our group is also participating in a Madrid Community project entitled “PrimPol: una nueva DNA Primasa/Polimerasa con un posible papel en envejecimiento” ([http://vmbacterio.cbm.uam.es/primpol/Primpol/Inicio.html](http://vmbacterio.cbm.uam.es/primpol/Primpol/Inicio.html))
Regulation of gene expression in *Leishmania*

**Figure 1.** Subcellular location of PrimPol in *L. major* promastigotes. The construct coding for LMPP2::mCherry was integrated into the genome by using the vector pLEXSY. k, kinetoplast; n, nucleus.
Regulation of gene expression in *Leishmania*

**Group Leader:**
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(Pontificia Universidad Javeriana, Bogotá, Colombia)
Regulation of gene expression in *Leishmania*

**Publications**


We have continued with the study of the mechanism of ø29 DNA replication initiated by TP-priming. We have optimized the ø29 replication origins for TP-primed initiation of replication. Ø29 DNA polymerase Lys529 is involved in the stabilization of the primer-terminus in the polymerization active site. This lysine and Glu233 of the TP establish contacts for the initiation of TP-DNA replication. The LExE motif, conserved in eukaryotic-type DNA polymerases, is involved in the interaction with the incoming nucleotide. Residues Tyr226 and Tyr390 in the ø29 DNA polymerase active site are involved in the mechanism of translocation and in dNTP and pyrophosphate binding. In the ø29 TP there is a correlation between the capacity of DNA binding and nucleoid localization. On the other hand, the nuclear and nucleoid localization of the TPs of ø29 and other bacteriophages are independently conserved functions. The ø29 protein p1 associates with the FtsZ ring of the Bacillus subtilis divisome producing an increase in the cell length and in the accumulation of the viral DNA. In collaboration with Dr. Beatriz González we have obtained the three-dimensional structure of the B. subtilis uracil-DNA glycosylase (UDG) free and in complex with the ø29 protein p56 and we have determined key amino acids for the UDG inhibition by p56.

The function of African swine fever virus genes in virus replication is being studied by the generation of virus recombinants. We have constructed a virus recombinant inducible in gene R298L coding for a viral protein kinase (PK), showing that under repression conditions the virus does not exit from the cell, although infectious intracellular virus is produced. The virus can exit the cytoplasmic viral factory but remains retained at the plasma membrane.
Reproduction of bacteriophage ø29
DNA African Swine Fever Virus

Figure 1. Three-dimensional structure of the UDG-p56 complex

Figure 2. Colocalization of ø29 protein p1 and B. subtilis protein FtsZ
Replication of bacteriophage ø29
DNA African Swine Fever Virus

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Replication of bacteriophage ø29
DNA African Swine Fever Virus

Publications (1)


Replication of bacteriophage ð29
DNA African Swine Fever Virus

Publications (2)


Replication of bacteriophage ø29
DNA African Swine Fever Virus

Other Activities

• Co-director of the Course “Nuevas perspectivas en Biomedicina” of the “Escuela de Biología Molecular Eladio Viñuela”. International University Menéndez Pelayo (2013).

• President of the organizing Committee of the XXXVI Congress of the Spanish Society of Biochemistry and Molecular Biology (SEBBM) (2013).

• Organizer of the XI Science Week at the Llurca Town hall. Asturias (2013).

• Co-director of the Course “Genome Stability: Replication, Repair and Mutagenesis” at the Master of Molecular and Cellular Biology in the Official Postgraduate Programme “Molecular Biosciences” at the Madrid Autonomous University (2013-14).

• Co-director of the Course “Retos en Biomedicina molecular en la segunda década del siglo XXI” of the “Escuela de Biología Molecular Eladio Viñuela”. International University Menéndez Pelayo (2014).

• Prizes and Distinctions

  • Madri+d 2012 Prize to the best patent granted by the Madri+d Foundation for Knowledge (2013).

  • Honour Member of the Scientific Senatum of the Sciences Faculty at Zaragoza University (2013-).

  • Honorary Professor at the Department of Molecular Biology of Madrid Autonomous University (2013-2014).

  • Prize to the “Excelencia Química” granted by the “Consejo General de Colegios Oficiales de Químicos” of Spain (2014).
Replication of bacteriophage ø29
DNA African Swine Fever Virus

Doctoral Theses

Madrid Autónoma University. Directors: Margarita Salas Falgueras y Daniel Muñoz Espín.
Our group studies the mechanisms by which eukaryotic cells prevent genomic instability, an important cause of aging and diseases such as cancer. We mainly study how genome integrity is maintained during chromosome replication, especially under conditions of DNA damage or replicative stress. The basic aspects of these processes are evolutionarily conserved, which allows us to use the budding yeast *Saccharomyces cerevisiae* as a model organism.

During this period we have continued the study of the function and regulation of the RAD6/RAD18 pathway of DNA damage tolerance. We have found that Rad5^{HTLF/SHPRH}, which mediates the error-free branch of this pathway, has a major role in the response to DNA damage during replication. Rad5 is required for the progression of replication forks through MMS-damaged DNA, reaches maximum levels during S phase and forms foci in the presence of DNA damage. Rad5 ensures the completion of chromosome replication under DNA-damaging conditions, reducing the risk of mutagenesis and thereby contributing to genome integrity maintenance.

We have also expanded the study of the Mus81-Mms4^{EME1} endonuclease, showing that it is required for the completion of chromosome replication and for cell survival when the DNA is damaged. We have found that Mus81-Mms4 gets activated at the end of S-phase and that it executes its function after the bulk of genome replication has finished. This mode of action prevents Mus81-Mms4 action during S phase and therefore the potential genomic instability derived from its nucleolytic function. At the same time, it is an efficient fail-safe mechanism for processing DNA intermediates that can persist after replication and need to be resolved before mitosis.
Chromosome replication and genome stability

Figure 1. Schematic illustration of the processes triggering the DNA damage tolerance response.

Figure 2. The Rad5 protein accumulates and forms nuclear foci in response to the presence of DNA damage.
Chromosome replication and genome stability

Group Leader:
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Alberto Jiménez Martín
Mª Ángeles Ortiz Bazán
Chromosome replication and genome stability

Publications


Chromosome replication and genome stability

Doctoral Theses

María Ángeles Ortiz Bazán (2014). Análisis del papel de los componentes de la ruta RAD6/RAD18 de Saccharomyces cerevisiae en la tolerancia al daño en el DNA durante la replicación cromosómica.
Universidad Autónoma de Madrid. Director: José Antonio Tercero.
Repair of bacterial DNA group

Research Summary

Our main objective is to get insights on the molecular mechanisms responsible for maintaining genetic information in bacteria, by functional analysis of purified repair proteins from the bacterium *Bacillus subtilis* whose vegetative cells and spores have to deal with DNA damage induced by extreme environmental conditions.

During these two years we have analyzed in vitro the functional properties of *B. subtilis* Ligase D (BsuLigD) and Ku (BsuKu) that constitute a minimal nonhomologous end joining (NHEJ) system in this bacterium. Our results have shown that the essential biochemical signatures exhibited by BsuLigD agree with its proposed function in NHEJ: 1) inherent polymerization activity showing preferential insertion of NMPs, 2) specific recognition of the phosphate group at the downstream 5´ end, 3) intrinsic ligase activity, 4) ability to promote realignments of the template and primer strands during elongation of mispaired 3´ ends, and 5) it is recruited to DNA by BsuKu that stimulates the inherent polymerization and ligase activities of the enzyme allowing it to deal with and to hold different and unstable DNA realignments.

Additionally, we have shown that BsuKu, along with its pivotal role in allowing joining of two broken ends by BsuLigD, is endowed with an AP/deoxyribose 5´-phosphate (5´-dRP)-lyase activity that can act on ssDNA, nicked molecules and DNA molecules without ends. Coordination with BsuLigD makes this protein able to cooperate in processing of AP sites during the NHEJ pathway. Our results showed that this activity is not restricted to *B. subtilis* as is also present in the Ku protein of the phylogenetically distant bacterium *Pseudomonas aeruginosa*, allowing us to expand our observations to other bacterial members provided with an NHEJ system.
Figure 1. End joining of partially complementary DNA ends with near terminal AP sites. Left panel, schematic representation of the end-joining reaction, indicating the different substrates (filled circle represents an abasic site), products and the catalytic reactions that take place. (A) The end joining reaction requires the previous processing of the AP sites by Ku and further ligation by LigD. (B) End joining with a downstream DNA molecule bearing a protruding 5’-dRP end.
Repair of bacterial DNA group

Group Leader: Miguel de Vega José

Postdoctoral fellows: Olga Zafra Amorós

Becarios predoctorales: Ana de Ory López
Repair of bacterial DNA group

Publications


Repair of bacterial DNA group

Other Activities

- IX Award Madrid+d to the best patent “Chimeras of phage ø29 DNA polymerase”