CAPÍTULO 1

Molecular and Cellular Biology

Luis Enjuanes
Coronavirus Laboratory

Mariano Esteban
Poxvirus and vaccines

Lluis Montoliu
Animal models by genetic manipulation

José Ramón Naranjo
Functional analysis of transcriptional repressor DREAM

Amelia Nieto
Mechanism of interaction between the influenza virus and the infected cell

Juan Ortín
Transciption and Replication of influenza Virus RNA

Dolores Rodríguez Aguirre
Molecular characterización of toroviruses

Jose F. Rodríguez-Aguirre
Coronavirus Laboratory

Carlos Mª Suñé Negre
mRNA Formation and Function

Antonio Alcamí (adscripción temporal desde marzo 2004)
Viral Modulation of the Immune Response

REPLICATION, VIRUS-HOST INTERACTION AND PROTECTION IN CORONAVIRUSES

Luis Enjuanes

Summary

The coronaviruses are single-stranded positive-sense RNA viruses with genomes of around 30 kb, responsible for infections of the respiratory and enteric mucosal tissues. Coronaviruses have high impact on animal and human health. Our group is interested in the molecular basis of replication and transcription, assembly, and virus-host interaction using transmissible gastroenteritis coronavirus (TGEV) and the severe and acute respiratory syndrome virus (SARS-CoV) as models. The information derived from these studies is being applied to the engineering of coronavirus based vectors.

Both virus replication and transcription, and virus-host interactions are mediated by the binding of virus RNA motifs to virus and host proteins and by protein-to-protein interactions. We have postulated that coronavirus transcription and replication involve 5' and 3' genome ends interaction, and the potential proteins involved in this process are being identified. Coronavirus transcription requires discontinuous RNA synthesis to link subgenomic RNA leader to coding sequences, a process similar to high copy-choice similarity-assisted RNA recombination. Based on a large amount of data generated by reverse genetics, our laboratory has proposed a transcription mechanism. We have proven that baseparing between the nascent RNA chain and the leader regulates the amount or subgenomic RNA produced. Nevertheless, there are additional regulatory mechanisms that influence the amount of subgenomic RNA, such as RNA-protein interactions. A major target of our program is to study the role of RNA chaperones in template switch during viral RNA synthesis.

In our second major area of work, virus-host interaction, we have postulated that specific virus structural proteins, such as the virus nucleoprotein that has been found in the cell

nucleus, and non-essential viral proteins, modulate these interactions. Using reverse genetic approaches, based on two infectious cDNA clones produced in our laboratory for TGEV and SARS-CoV, we are studying the influence of coronavirus genes on virus attenuation, cell cycle, and on relevant host functions such as immune response. Comparative genomic and proteomic information is essential in these studies.

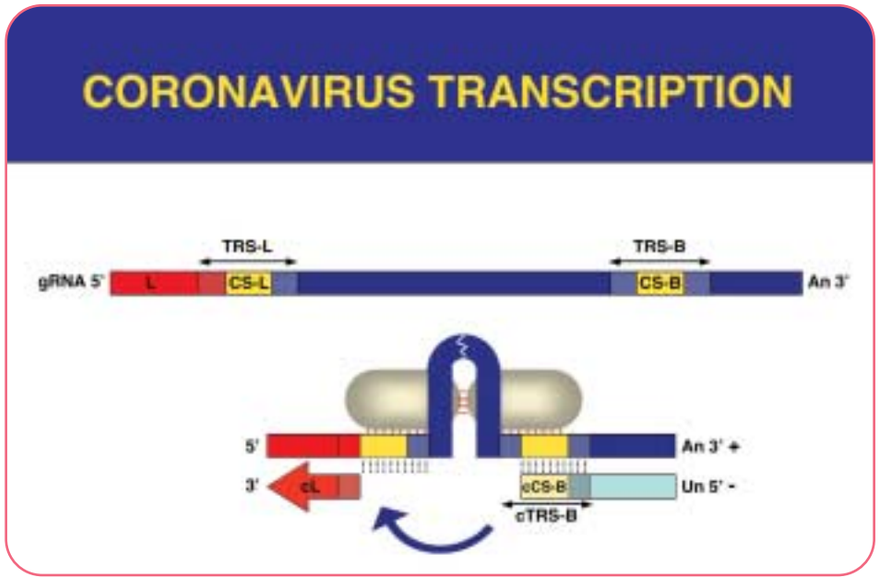

Figure 1. Diagram of the elements involved in coronavirus transcription. In the top bar, the sequence elements involved in the discontinuous synthesis of the negative RNA strand. CS-L and CS-B, leader and body CSs. TRS-L and TRS-B, transcription regulating sequences from leader and body. An, Poly A. In the lower part of the figure, an scheme of the discontinuous transcription during negative strand synthesis and of the sequence elements involved is represented. CS-B and cTRS-B represents the CS-B and cTRS-B complementary sequences, respectively. Un, Poly U. C. Leader and body sequences are probably located in close proximity in higher order structures maintained by RNA-protein and protein-to-protein interactions.

PERSONNEL

Group Leader:
Luis Enjuanes

Postdoctoral Fellows:
Fernando Almazán
Isabel Sola
Javier Ortego
Sara Alonso
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Predoctoral Fellows:
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Carmen Capiscol
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Juan Ceriani
Aitor Nogales González

Technical Assistants:
Carlos M. Sánchez
Diana Dorado
Margarita González

Visitors:
Caroline Lassnig, Vienna, Austria.
Kersting Saenger, Riems, Germany.
Patricia Sabella, Fort-Dodge, Gerona, Spain.

PUBLICATIONS

Ortego, J., Sola I., Almazan F., Ceriani J. E., Riquelme, C., Balasch, M., Plana J. and Enjuanes, L. (2003). Transmissible gastroenteritis coronavirus gene 7 is not essential, but affects *in vivo* replication and virulence. *Virology*. **308**, 13-22.

Sola, I., Alonso, S., Zuñiga, S., Balach, M., Plana-Durán, J. y Enjuanes, L. (2003). Engineering transsmisible gastroenteritis virus genome as an expression vector inducing latogenic immunity. *J. Virol.* **77**, 4357-4369.

Holmes, K.V. and Enjuanes, L. (2003). The SARS coronavirus: a postgenomic era. *Science* **300**, 1377-1378.

Escors, D., Izeta, A., Capiscol, C., Enjuanes, L. (2003). Transmissible gastroenteritis coronavirus packaging signal is located at the 5' end of the virus genome. *J. Virol.* **77**, 7890-7902.

González, J.M., Gómez-Puertas, P., Cavanagh, D., Gorbalenya, A.E. and Enjuanes. L. (2003). A comparative sequence analysis to revise the current taxonomy of the family *Coronaviridae*. *Arch. Virol.* **148**, 2207-2235.

Zuñiga, S., Sola, I., Alonso, S. and Enjuanes, L. (2004). Sequence motifs involved in the regulation of discontinuous coronavirus subgenomic RNA synthesis. *J. Virol.* **78**, 980-994.

Bailey, M., Haweson, K., Jones, P., Miller, B., Sola, I., Enjuanes, L. and Stokes, C.R. (2004). Effect of infection with transmissible gastroenteritis virus on concomitant immune responses to dietary and injected antigens. *Clin. Diagn. Lab. Immun.* **11**, 337-343.

Schwegmann-Weßels, C., Al-Falah, M., Escors, D., Wang, Z., Zimmer, G., Deng, H., Enjuanes, L., Naim, H.Y., Herrler, G. (2004). A novel sorting signal for intracellular localization is present in the S protein of a porcine coronavirus but absent from SARS-associated coronavirus. *J. Biol. Chem.* **279**, 43661-43666.

Escors, D., Capiscol, C. and Enjuanes, E. (2004). Immunopurifation applied to the study of virus protein composition and encapsidation. *J. Virol.* **119**, 57-64.

Enjuanes, L., Sola, I., Alonso, S. and Zuñiga, S. (2004). Coronavirus reverse genetics: viral based vectors for gene expression. Curr. Top. *Microbiol. & Immunol.* **287**, 161-197.

Almazan, F., Galan, C. and Enjuanes, L. (2004). The nucleoprotein is required for efficient coronavirus genome replication. *J. Virol.* **78**, 12683-12688.

Enjuanes, L. (2004). Diseño y bioseguridad de vacunas y vectores virales. In: La Real Expedición Filantrópica de la Vacuna. Doscientos Años de Lucha Contra la Viruela. Eds. Susana Ramírez, Luis Valenciano, Rafael Nájera, Luis Enjuanes. CSIC. Madrid, España, 347-381.

LIBROS / BOOKS

Enjuanes, L., Almazán, F and Ortego, J. (2003). Virus based vectors for gene expression in mammalian cells: coronavirus. In: "Gene Transfer and Expression in Mammalian Cells. Eds. S. C. Makrides. Elsevier Science. Amsterdam. Holanda.

Enjuanes, L. (2004). Coronavirus replication and reverse genetics. Current Topics in Microbiology and Immunology. Springer Verlag. Heidelberg, Germany.

Walker, P.J., Bonami, J. R., Boonsaeng, V., Chang, P.S., Cowley, J.A., Enjuanes. L., Flegel, T. W., Lightner, D. V., Loh, P. C., Snijder, E.J., Tang, K. (2004). Eight Report of the International Committee on Taxonomy of Viruses Roniviridae. In: Virus Taxonomy. Classification and Nomenclature of Viruses. Academic Press. San Diego. USA.

Enjuanes, L. (2004). La Real Expedición Filantrópica de la Vacuna. Doscientos Años de Lucha Contra la Viruela. Eds. S. Ramírez, L. Valenciano, R. Nájera, L. Enjuanes. CSIC. Madrid, Spain.

ARTICULOS DE DIVULGACIÓN / DIVULGATION ARTICLES

Escors, D., Enjuanes, L. (2003). Las enfermedades virales emergentes: el coronavirus del sindorme respiratorio agudo y severo. Historia Natural. Vol 1. PP. 48-52. October.

Enjuanes, L. (2004). Vacunacion y bioseguridad. "La Real Expedicion Filantropica de la Vacuna. Doscientos Años de Lucha Contra la Viruela". CSIC. Madrid. Spain. November.

RESEARCH PROJECTS

Enjuanes, L.
Immunotherapy of enteric infections by rotaviruses and coronaviruses using plantibodies.
Organismo financiador: UE, Proyecto: Ref. CE: QLK2-CT-2000-00739; CSIC: LIFE/992/0587, 2000 - 2003.

Enjuanes, L.(Coordinador del proyecto)
Biosafe coronavirus vaccine vector for the prevention of human infections of the enteric and respiratory tracts.
Organismo financiador: UE, Proyecto: Ref. CE: QLK2-CT-2001-00874; CSIC: LIFE/992/0859, 2001 – 2005.

Enjuanes, L.
Biosafe coronavirus vector-based vaccine for prevention of foot-and-mouth disease.
Organismo financiador: UE, Proyecto: Ref. UE: QLK2-CT-2002-00825, CSIC: LIFE/001/1282, 2002 – 2006.

Enjuanes, L.
Targeting immunostimulating complex production in plants.
Organismo financiador: UE., Proyecto: Ref. UE: QLK2-CT-2002-01050, CSIC: LIFE/001/1273, 2002 – 2005.

Enjuanes, L.
Ingeniería de genomas de coronavirus para el diseño de vectores bioseguros.
Organismo financiador: CICYT, Proyecto: Ref. BIO2001-1699, Duración: 2001 – 2004.

Enjuanes, L.(Coordinador del proyecto)
Development of intervention strategies against SARS in a European-Chinese taskforce.
Organismo financiador: UE, Proyecto: Ref. UE: SP22-CT-2004-511060, CSIC:SSP/STREP/02/0324, 2004 – 2007.

Enjuanes, L.
Replicación, interacción virus-huesped y protección en coronavirus.
Organismo financiador: CICYT, Proyecto: Ref. BIO2004-00636, 2004 – 2007.

Enjuanes, L.
Vectores virales y no virales en terapia génica. Aplicación de sistemas poliméricos inteligentes para la formación de complejos de baja toxicidad.Organismo financiador: CSIC, Proyecto: Ref. 200420F0294, 2004 – 2005.

Enjuanes, L.
Genomic inventory, forensis markers, and assessment of potential therapeutic and vaccine targert for viruses relevant in biological crime and terrorism.
Organismo financiador: UE, Proyecto: FP6-2004-SSP-4, 2004 – 2007.

DOCTORAL THESES

Cristina Riquelme Gabriel. (1998-2004).
Vectores basados en genoma del coronavirus TGEV.
Universidad Autonoma de Madrid. Madrid.

Carmen Galán Avella. (2002-05).
Estudio de la replicación del coronavirus TGEV.
Universidad Autonoma de Madrid. Madrid.

M. Carmen Capiscol Perez de Tudela. (2003-05).
Estudio de la señal de encapsidación del virus coronavirus TGEV.
Universidad Autonoma de Madrid. Madrid.

Juan E. Ceriani. (2003-05).
Construcción de partículas análogas a rotavirus (VLP's) utilizando vectores virales basados en genomas de coronavirus.
Universidad Autonoma of Barcelona. Barcelona.

José Luis Moreno. (2004-05).
Estudio de la regulación de la transcripción de coronavirus. Diseño de un vector viral para vacunas y terapia génica.
Universidad Autonoma de Madrid. Madrid.

Marta L. De Diego. (2004-05).
Interacción del coronavirus SARS con el huésped y protección frente a infecciones producidas por este virus
Universidad Autonoma de Madrid. Madrid.

Aitor Nogales González. (2005).
Proteinas virales y celulares implicadas en la replicación del coronavirus TGEV.
Universidad Autonoma de Madrid. Madrid.

PATENTS

Enjuanes, L., Almazán Toral, F., González Martínez, J.M., Izeta, A., Pénzes, Z., Alonso Villanueva, S., Sánchez, C.M., Sola Gurpegui, I.,Sánchez Morgado, J.M., Calvo Alcocer, E., Ortego Alonso, J., Escors, D., Barrado Guerrero, P., Riquelme Gabriel,C. y Plana Durán, J.
Nº Registro: CECT 5265(P 9902673).

POXVIRUS Y VACUNAS

Mariano Esteban

Summary

The aims of our group are geared to understand molecular basis in the pathogenesis of infectious agents and their interaction with the host, as well as to use this knowledge in the development of vaccines effective against diseases like AIDS, malaria and leishmaniasis.

As a model system of infectious agent and as a delivery vector for the expression of genes of interest, we used vaccinia virus (VV) a member of the poxvirus family.

We focus our research in three main areas of interest:

1. Vaccinia virus assembly.
2. Virus-host cell interactions and action of interferons;
3. Developmen of vaccines against Aids, malaria and leishmaniasis.

We would like to respond to the following challenging questions:

a) what is the structure of the different forms of vaccinia virus (VV) during morphogenesis and how these forms contribute to virus infection to cells and tissue distribution.

b) how VV gets into cells and what are the viral components involved

c) what is the structure of the viral complex A27L/A17L involved in virus attachment to cells.

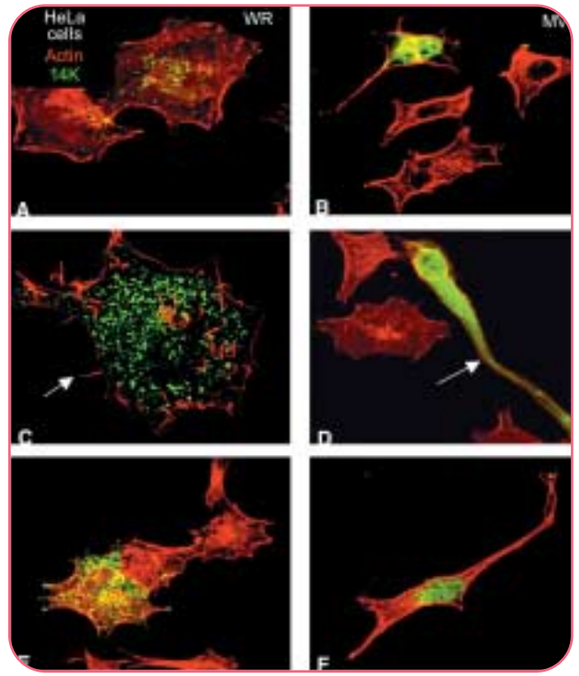

Figure 1. Gene expression of MVA in human cells.

d) how VV turns-off host cell translation

e) what is the impact of VV and its mutant viruses on host cell gene expression profiling and how some of the cellular genes facilitate or inhibit VV replication.

f) What is the role of interferon (IFN)-induced genes (i.e, PKR and the 2-5A synthetase/RNase L system) on antiviral and anticellular functions, how viruses evade the IFN system and can these viruses and/or the IFN-induced genes be used to destroy tumour cells.

g) Can we modulate the immune system (humoral and cellular) with poxvirus vectors and generate effective vaccines against relevant human diseases like AIDS, malaria, leishmaniasis, HCV and cancer.

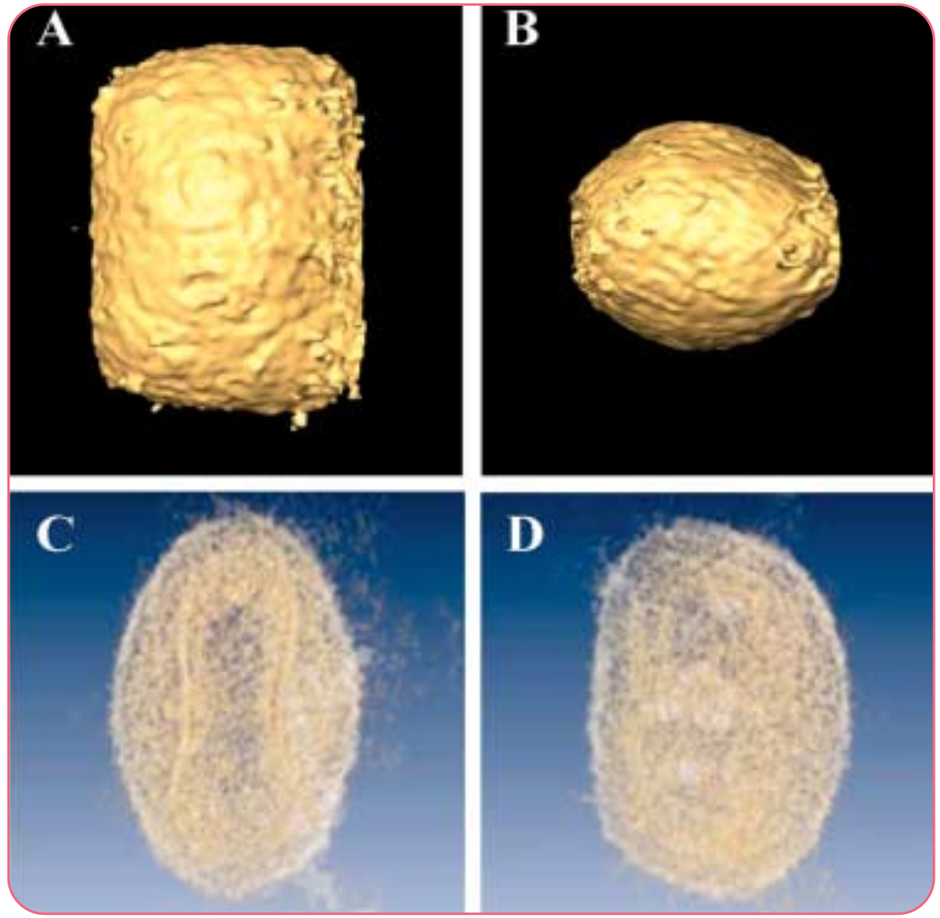

Figure 2. The structure of the infectious form (IMV) of vaccinia virus (VV) has been defined at the resolution of 4-6 nm through cryo-electron tomography.

PERSONNEL

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PUBLICATIONS

Gherardi, M.M., Nájera, J.L., Pérez-Jiménez, E., Guerra, S., García-Sastre, A. and Esteban, M. (2003). Prime/boost immunization schedules based on influenza and vaccinia virus (VV) vectors (MVA and WR) potentiate cellular immune responses against HIV-env protein systemically and in the genito-rectal draining lymph nodes. *J. Virol.* **77**, 7048-7057.

Guerra, S., López-Fernández, L., Pascual-Montano, A., Muñoz, M., Harsman, K. and Esteban, M. (2003). Cellular gene expression survey upon vaccinia virus infection of human HeLa cells. *J. Virol.* **77**, 6493-6506. (front cover Dec. issue).

Gallego-Gómez, J.C., Risco, C., Rodríguez, D., Cabezas, P., Guerra, S., Carrascosa, J.L. and Esteban, M. (2003). Differences in virus-induced cell morphology and in virus maturation between the MVA and other strains (WR, Ankara, NYCBH) of vaccinia virus in infected human cells. *J. Virol.* **77**, 10606-10622.

Gil, J., García, M.A., Gómez-Puertas, P., Guerra, S., Rullás, J., Alcamí, J. and Esteban, M. (2004). TRAF family proteins link PKR with NF-kB activation. *Mol. Cell. Biol.* **24**, 4502-4512.

Gherardi, M.M., Pérez-Jimenez, E., Nájera, J.L. and Esteban, M. (2004). Induction of HIV immunity in the genital tract after intranasal delivery of a MVA vector: enhanced immunogenicity after DNA prime-modified vaccinia virus Ankara boost immunization schedule. *J Immunol.* **172(10)**, 6209-20.

Tapia, E., Pérez-Jimenez, E., Lopez-Fuertes, L., Gonzalo, R. and Esteban, M (2003). The combination of vectors expressing IL-12+IL-18 elicits high protective immune response against cutaneous leishmaniasis after priming with DNA-p36/LACK and the cytokines, followed by a booster with a vaccinia virus recombinant expressing p36/LACK. *Microbes and Infection* **5**, 73-84.

Ramirez, J.C., Finke, D., Esteban, M., Kraehenbuhl, J.P. and Acha-Orbea, H (2003). Tissue distribution of Modified Vaccinia Virus Ankara (MVA) after mucosal or systemic administration. *Arch. Virol.* **148**, 827-839.

Gherardi, M.M., Nájera, J.L., Pérez-Jiménez, E., Guerra, S., García-Sastre, A. and Esteban, M. (2003). Prime/boost immunization schedules based on influenza and vaccinia virus (VV) vectors (MVA and WR) potentiate cellular immune responses against HIV-env protein systemically and in the genito-rectal draining lymph nodes. *J. Virol* **77**, 7048-7057.

Ramiro, M.J., Zárate, J.J., Hanke, T., Rodríguez, D., Rodríguez, J.R., Esteban, M., Lucientes, J., Castillo, J.A. and Larraga, V (2003). Protection against Leishmania infantum visceral leihsmaniasis in dogs is achieved by immunization with heterologous prime-booster regime using LACK-expressing DNA and recombinant vaccinia virus. *Vaccine* **21**, 2471-2484.

Guerra, S., López-Fernandez, L., Pascual-Montano, A., Muñoz, M., Harsman, K. and Esteban, M (2003). Cellular gene expression survey upon vaccinia virus infection of human HeLa cells. *J. Virol* **77**, 6493-6506. (front cover Dec issue).

Esteban, M., García, M.A., Domingo-Gil, E., Arroyo, J., Nombela, C. and Rivas, C. (2003). The latency protein LANA 2 from Kaposi´s sarcoma associated herpesvirus (KSHV) inhibits apoptosis induced by PKR but not RNase L activation. *J. Gen.Virol* **84**, 1463-1470.

Gherardi, M.M., Ramirez, J.C. and Esteban, M (2003). Interleukin-12 (IL-12) and IL-18 synergize to clear vaccinia virus infection: involvement of innate and adaptive components of the immune system. *J. Gen. Virol.* **84**, 1961-1972.

González-Lopez, C., Martinez-Costas, J., Esteban, M. and Benavente, J (2003). Avian reovirus sA protein is an inhibitor of the double-stranded RNA-dependent protein kinase PKR. *J. Gen. Virol.* **84**,1629-1639.

Gallego-Gómez, J.C., Risco, C., Rodríguez, D., Cabezas, P., Guerra, S., Carrascosa, J.L. and Esteban, M (2003). Differences in virus-induced cell morphology and in virus maturation between the MVA and other strains (WR, Ankara, NYCBH) of vaccinia virus in infected human cells. *J. Virol* **77**, 10606-10622.

González-Aseguinolaza, G., Nakaya, Y., Molano, A., Dy, E., Esteban, M., Rodríguez, D., Rodríguez, J.R., Palese, P., García-Sastre, A. and Nussenzweig, R.S (2003). Induction of protective immunity against malaria by prime/boost immunization with recombinant cold- adapted influenza and modified vaccinia virus Ankara viruses expressing a CD8+ T cell epitope derived from the cincumsporozoite protein of *Plasmodium yoelii. J. Virol* **77** , 11859-11866.

Gómez, C.E., Abaitua, F., Rodríguez, D., Duarte, C. and Esteban, M (2003). Efficient CD8+ T cell response to HIV-env V3

loop epitope from multiple isolates by a DNA prime/vaccinia virus boost (rWR and rMVA strains) immunisation regime and enhancement by the cytokine IFN-γ. Virus Research 105, 11-22

Diderlaurent, A., Ramirez, J.C., Graff, M., Gherardi, M., Orbea, H-A., Wagner, H., Esteban, M., Kraehenbuhl, J-P, and Sirard, J-C (2004). Attenuated poxviruses expressing a synthetic HIV protein stimulate HLA-A2 restricted cytotoxic T cell responses. *Vaccine* **22**, 3395-3403.

Gil, J., García, M-A., Gómez-Puertas, P., Guerra, S., Rullás, J., Alcamí, J. and Esteban, M (2004). TRAF family proteins link PKR with NF-kB activation. *Mol. Cell. Biol.* **24**, 4502-4512.

Guerra, S., Lopez-Fernandez, L.A., Conde, R., Pascual-Montano, A., Harshman, K. and Esteban, M. (2004). Microarray Analysis Reveals Characteristic Changes of Host Cell Gene Expression in Response to Attenuated Modified Vaccinia Virus Ankara Infection of Human HeLa Cells. *J Virol* **78(11)**: 5820-34.

Gherardi, M. M., Pérez-Jimenez, E., Nájera, J.L and Esteban, M. (2004). Induction of HIV immunity in the genital tract after intranasal delivery of a MVA vector: enhanced immunogenicity after DNA prime-modified vaccinia virus Ankara boost immunization schedule." *J Immunol* **172(10)**: 6209-20.

Esteban, M. (2004). Conceptos y futuras aplicaciones de la genómica, proteómica y bioinformática en el campo de la salud. En Genoma España, *Salud Humana*, pp 99-104

Esteban, M. (2004). Desarrollo de nuevas vacunas basadas en poxvirus. En "Real Expedición Filantrópica de la Vacuna. Doscientos años de lucha contra viruela". Biblioteca de Historia de América, CSIC. p 333-345.

Gil, J. and Esteban, M. (2004). Vaccinia virus recombinants as a model system to analyze interferon-induced pathways. *J. Interferon and Cytokine Research* **24**, 637-646

RESEARCH PROJECTS

Mariano Esteban. Principal Spanish Investigator.
Effector and memory anti-malaria CD8+ cell responses.
National Institutes of Health (NIH), 1 RO1 AI44375-01, 1999-2003, US $165.000.

Mariano Esteban. Principal Spanish Investigator.
Visceral Leishmaniasis vaccine: murine model studies.
National Institute of Health (NIH), USA. RO1 AI45044. 2000-2003.US $ 50.000

Project Leader of the EuroVac Cluster, European Vaccine Effort Against HIV/AIDS, Fifth Framework Programme, QLRT-PL1999-01321, Euros 329.065, 1999-2004.

Concerted Action, Fifth Framework Programme, European Vaccine against Aids (EVA) CFAR, QLRT-PL1999-00609, 2000-2003.

Mariano Esteban. Principal Investigator.
Contract wih GALENICA , USA, 2003-2004.

Mariano Esteban. Principal Investigator.
Premio IBERDROLA Ciencia y Tecnología, Profesores Visitantes, 2000-2003.

Mariano Esteban. Principal Investigator.
Desarrollo de nuevas herramientas moleculares para el estudio del virus de la hepatitis C y su aplicación a morfogénesis, estructura, resistencia del virus a interferon y caracterización de la respuesta inmune al virus.
BIO2000-0340-P4, 2001-2003. 171.649 Euros.

Mariano Esteban. Principal Investigator.

Diseño y utilización del virus vaccinia como vacuna contra distintas enfermedades: análisis de la interacción virus-célula y modulación de la respuesta inmune.
BIO2001-2269, 2001-2003, 170.000 Euros.

Mariano Esteban. Principal Investigator.
Desarrollo de nuevas herramientas moleculares para el estudio del virus de la hepatitis C y su aplicación a morfogénesis, estructura, resistencia del virus a interferon y caracterización de la respuesta inmune al virus.
BIO2000-0340-P4. 2000-2003, 171.649 Euros.

Mariano Esteban. Principal Investigator.
Mecanismo de acción de los interferones: análisis structural y funcional de la proteína quinasa PKR, un activador de apoptosis e inhibidor viral.
BMC2002-03246, 2002-2005, 196.650 Euros.

Mariano Esteban. Principal Investigator.
Analysis of the molecular mechanism of hepatitis C virus (HCV) resistance to antiviral therapy.
EU QLK2-CT-2002-00954. 2002-2005, 124.313 Euros.

Mariano Esteban. Coordinator.
Increasing the potency of vaccinia MVA vaccines.
EU QLK2-CT-2002-01867. 2002-2005. 220.000 Euros.

Mariano Esteban. Principal Investigator.
Potenciación de la respuesta inmune (sistémica y de mucosas frente al virus de la inmunodeficiencia humana (VIH-1).
FIPSE, 2002-2005, 209.365 Euros.

Mariano Esteban. Principal Investigator.
Vaccine strategies for combined targeting of innate and adaptive immune pathways (VaccTIP).
EU-2004-012161. 177.000 euros. 2004-2006.

Mariano Esteban. Principal Investigator.
Diseño de nuevas vacunas tanto preventivas como terapéuticas para las enfermedades de mayor prevalencia: sida, hepatitis C y cáncer de próstata.
BIO2004-03954,180.000 euros. 2004-2007.

Mariano Esteban. Principal Investigator.
Desarrollo de vacunas contra enfermedades prevalentes.
Fundación Botín, 200.000 euros/year. 2005-2010.

Mariano Esteban. Principal Investigator.
Desarrollo de una vacuna contra Leishmaniasis.
Comunidad de Madrid. 41000 euros. 2005.

Mariano Esteban. Principal Investigator.
Contract with GRIFOLS. 2005-2006.

DOCTORAL THESES

Juan Carlos Gallego Gómez (2003).
Biología celular de la infección y morfogénesis de mutantes atenuados del virus vaccinia.
Universidad Autónoma de Madrid. Sobresaliente *cum laude*.

Carmen E. Gómez (2003).
Respuesta inmune generada por sistemas combinados de vacunación frente a péptidos de la envuelta del VIH-1 incluidos en la proteína multiepitópica TAB-13.
Universidad Autónoma de Madrid. Sobresaliente *cum laude*.

María Angel García Chaves (2004).
Mecanismo de acción y regulación de la proteína quinasa inducida por interferon, PKR.
Universidad Autónoma de Madrid. 30 Abril de 2004. Sobresaliente *cum laude*. Premio Extraordinario UAM.

CONTRACTS

Empresas:

Analisis de anticuerpos contra el virus vaccinia en preparados de inmunoglobulinas humanas (IGIVs).
GRIFOLS, S.A , 2004-2006.

Fundaciones:

Principal investigator.
Potenciación de la respuesta inmune (sistémica y de mucosas) frente al virus de la inmunodeficiencia humana (VIH-1).
FIPSE, 2002-2006.

Principal Investigator.
Desarrollo de vacunas contra enfermedades prevalentes.
Fundación Botín, 2005-2010.

PATENTS

Pérez-Jiménez, E. y Mariano Esteban, M.
VECTORES RECOMBINANTES BASADOS EN EL VIRUS MODIFICADO DE ANKARA (MVA) COMO VACUNAS CONTRA LEISHMANIASIS.
Solicitud de invención Nº 200501886.

Gómez, C.E., Nájera, J.L., Jiménez, V. y Esteban, M.
VECTORES RECOMBINANTES BASADOS EN EL VIRUS MODIFICADO DE ANKARA (MVA) COMO VACUNAS PREVENTIVAS Y TERAPEUTICAS CONTRA EL SIDA.
Solicitud de invención Nº 200501841.

ANIMAL MODELS BY GENETIC MANIPULATION

Lluis Montoliu

Summary

We are interested in understanding how mammalian expression domains work and how they are organised within genomes.

We would like to know the required regulatory elements that identify a given expression domain and specify its expression pattern in space, time and level, to improve the dessign of gene transfer strategies, used for animal transgenesis and for gene therapy.

We use two experimental models: the mouse tyrosinase and whey acidic protein genes, two independent developmentally regulated and tissue-specific loci that have served us to identify a number of key regulatory elements, including boundaries and LCRs.

We address our experiments *in vivo*, using transgenic mice carrying large chromosomal-type constructs that we engineer by homologous recombination to investigate the functional role of specific sequences, and *in vitro*, using cells and chromatin DNA-protein analyses. Further, our laboratory generates and analyses new animal models of human diseases by genetic modification of mice.

We have used a number of tyrosinase transgenic mice to address the retinal deficits commonly associated with albinism, the normal mammalian retinal development and the genes involved in the process.

Finally, through collaborations, we have generated a number of additional animal models (transgenics and knockouts) for CNS-related diseases or conditions (Alzheimer, pain, psychosis).

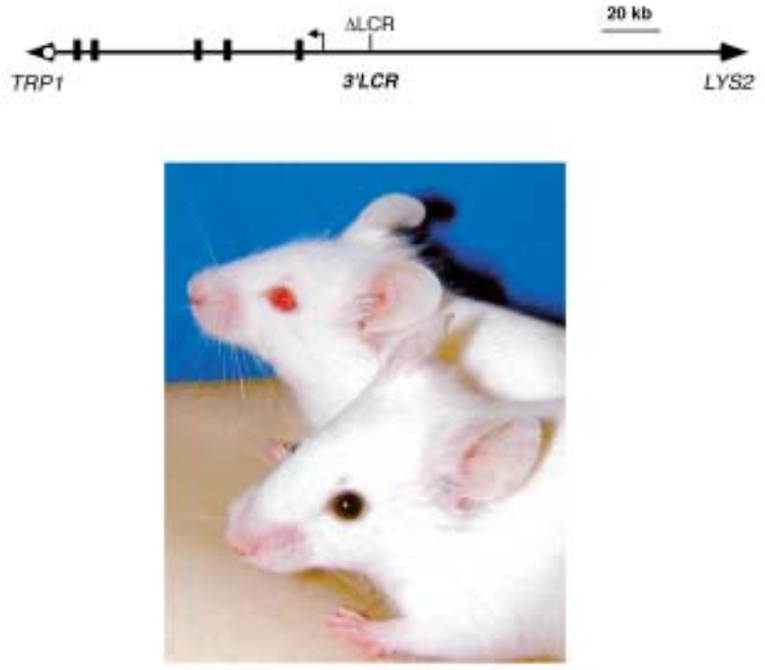

Figure 1. Albino and transgenic mouse (in front) generated by ICSI with a YAC carrying the tyrosinase gene with a deleted LCR prepared by homologous recombination in yeast cells. A severe decrease of tyrosinase expression is seen in the skin but not in the eye (see Moreira et al. 2004).

PERSONNEL

Laboratory Picture (December 2004) From left to right: Lluís Montoliu, Alfonso Lavado, Victoria Tovar, Lucía Regales, Julio Pozueta, Rosa Roy (in front), Soledad Montalbán, Marta Cantero, Ángel García, Patricia Cozar.

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Dra. Mª Carmen Muñoz (Trasngenic Unit, Parc Científic de Barcelona, PCB) (July-August 2004)
Dr. Glen Jeffery (University College London, Institute of Ophthalmology, London, UK) (January and December 2004)

PUBLICATIONS

INTERNATIONAL SCIENTIFIC JOURNALS

Moreira, P.N., Giraldo, P., Cozar, P., Pozueta,. J, Jiménez, A., Montoliu, L.* and Gutiérrez-Adan A*.(2004). Efficient generation of transgenic mice with intact yeast artificial chromosomes by intracytoplasmic sperm injection. *Biology of Reproduction* Dec;**71(6)**:1943-7.

Montoliu, L. (2004). 5th Transgenic Technology meeting (http://www.imbim.uu.se/transtech) Transgenic Research 13:605-6. [meeting report]

Giménez, E., Lavado, A., Giraldo, P., Cozar, P., Jeffery, G., Montoliu, L. (2004). A transgenic mouse model with inducible Tyrosinase gene expression using the tetracycline (Tet-on) system allows regulated rescue of abnormal chiasmatic projections found in albinism. *Pigment Cell Research* Aug;**17(4)**:363-70.

Montoliu, L., Larue, L. and Beermann, F. (2004). On the use of regulatory regions from pigmentary genes to drive the expression of transgenes in mice. *Pigment Cell Research* Apr;**17**(2):188-90.

Regales, L., Giraldo, P., García-Díaz, A., Lavado, A. and Montoliu, L. (2003). Identification and functional validation of a 5' upstream regulatory sequence in the human tyrosinase gene homologous to the locus control region of the mouse tyrosinase gene. *Pigment Cell Research* Dec;**16(6)**:685-92.

Giraldo, P., Rival-Gervier, S., Houdebine, L.M. and Montoliu, L. (2003). The potential benefits of insulators on heterologous constructs in transgenic animals. *Transgenic Research* Dec;**12(6)**:751-5.

Giménez, E., Lavado, A., Giraldo, P. and Montoliu, L. (2003). Tyrosinase gene expression is not detected in mouse brain outside the retinal pigment epithelium cells. *European Journal of Neurosciences* Nov;**18(9)**:2673-6.

Montoliu, L. (2003). Manipulating the mouse embryo: easier than ever! *Transgenic Research* **12**:635-6. [book review].

Giraldo, P., Martínez, A., Regales, L., Lavado, A., García-Díaz, A., Alonso, A., Busturia, A. and Montoliu, L. (2003). Functional dissection of the mouse tyrosinase locus control region identifies a new putative boundary activity. *Nucleic Acids Research* Nov **1;31(21)**:6290-305.

Langa, F., Codony, X., Tovar, V., Lavado, A., Giménez, E., Cozar, P., Cantero, M., Dordal, A., Hernández, E, Pérez R, Monroy, X., Zamanillo, D., Guitart, X. and Montoliu, L. (2003). Generation and phenotypic analysis of sigma receptor type I (sigma 1) knockout mice. E*uropean Journal of Neurosciences* **18(8)**:2188-96.

Millot, B., Montoliu, L., Fontaine, M.L., Mata, T. and Devinoy, E. (2003). Hormone-induced modifications of the chromatin structure surrounding upstream regulatory regions conserved between the mouse and rabbit whey acidic protein genes. *Biochemical Journal* **15;372(Pt 1)**:41-52.

Montoliu, L. (2003). Simple databases to monitor the generation and organisation of transgenic mouse colonies. *Transgenic Research* **12(2)**:251-3.

INTERNATIONAL BOOKS AND BOOK CHAPTERS

Montoliu, L. (2003). Large-scale preparation of agarose plugs of yeast DNA (pp. 326-328); Purification of YAC DNA with filtration units (pp- 329-331). Preparing injection buffer for BAC/YAC DNA (pp. 333). In: Manipulating the Mouse Embryo. A Laboratory Manual (Third Edition). Andras Nagy, Marian Gertsenstein, Kristina Vintersten, Richard Behringer (Eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Millot, B., Montoliu, L., Petitbarat, M., Mata, T., Fontaine, M.L. and Devinoy, E. (2003).Regulation of milk protein gene expression. In: Milk & Research (38º Simposio Internazionale di Zootecnia) G.F. Greppi & G. Enne (eds.). MG Print on Demad. Lodi, pp. 3-13.

NATIONAL BOOKS AND BOOK CHAPTERS

Montoliu, L. (2004). Clonación en mamíferos: aspectos científicos e implicaciones terapéuticas. En: Últimas investigaciones en biología: células madre y células embrionarias. José Fernández Piqueras (Ed.), Publicaciones de la Universidad Internacional Menéndez y Pelayo (UIMP), Ministerio de Educación y Ciencia. (pp. 55-88).

Montoliu, L. (2003). Trasiego de genes (animales transgénicos, mutantes y clónicos). En: 50 años de ADN. Pedro García Barreno (Ed.), Editorial Espasa-Forum, capítulo 6, (pag. 183-227), Madrid.

Montoliu, L. (2003). Células troncales: aspectos científicos. En: Células troncales humanas: aspectos científicos, éticos y jurídicos. Juan Ramón Lacadena (Ed.), Colección Dilemas Éticos de la medicina actual-17, Vol. 49 (pag. 23-62). Publicaciones de la Universidad Pontificia Comillas/Editorial Desclée de Brouwer, S.A.

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RESEARCH PROJECTS

Montoliu, Lluis
Functional and structural characterisation of genomic boundaries
Spanish Ministry of Science and Technology, National Plan R+D+i, Biotechnology Program, BIO2003-08196, 2004-2006.

Montoliu, Lluis (sub-project, Coordinator: Dr. Mara Dierssen, CRG, Barcelona)
Murine models of central nervous system (CNS) disease.
Autonomous Government of Catalunya, Generalitat de Catalunya, Thematic Network, 2003-2005, 2001-2003.

Montoliu, Lluis
Identification of genes associated with retinal development: analysis of visual deficits associated with hypopigmentary diseases (albinism).
Autonomous Government of Madrid, CAM, 08.5/0046.1/2003, 2003-2004.

Montoliu, Lluis (Austrian partner: Prof. Mathias Müller, Veterinary University of Vienna)
Artificial chromosomes in mammary gland transgenesis.
Joint Collaborative Project, Spain-Austria, HU2001-0025, 2002-2003.

Montoliu, Lluis
Genomic boundaries in gene transfer events.
Spanish Ministry of Science and Technology, National Plan R+D+i, Biotechnology Program, BIO2000-1653, 2001-2003.

DOCTORAL THESES

Mata González, Teresa
Functional and structural characterisation of new mammary gland-specific expression vectors based in the mouse whey acidic protein gene.
Centro de Investigaciones y Estudios Avanzados (CINVESTAV), Instituto Politécnico Nacional, México DF (2004). Mark: Apto
Supervisors: Dr. Vianney Ortiz-Navarrete (CINVESTAV, México, DF) and Dr. Lluís Montoliu.

Lavado Júdez, Alfonso Javier
Animal models for the functional study of the mouse tyrosinase gene and the consequences associated with its mutation in the mammalian visual system.
Autonomous University of Madrid (2004). Mark: Excellent cum laude.
Supervisor: Dr. Lluís Montoliu

CONTRACTS

Lluís Montoliu
Formation in techniques for the generation and the analysis of trasngenic and knockout mice.
Fundación Parc Científic de Barcelona (PCB), Barcelona Science Park Foundation (Barcelona), June-August 2004.

Lluís Montoliu
Generation of chimeras to obtain knockout mice for the LAT2 gene.
Fundación Institut de Recerca Oncològica (IRO), Institute of Oncology Research Foundation (Barcelona).
June-August 2004.

Lluís Montoliu
Production, rederivation and cryopreservation of knockout mice for the Sigma-I receptor gene.
Laboratorios del Dr. Esteve, S.A. (Barcelona).
July 2003- June 2006.

Lluís Montoliu
Analysis of genetic polymorphisms (microsatellite variants) in mice.
Bionostra, S. L. (Madrid).
May-July 2003.

Lluís Montoliu
Analysis of the phenotype of knockout mice for the Sigma-I receptor gene.
Laboratorios del Dr. Esteve, S.A. (Barcelona).
September 2001-August 2004.

PATENTS

Moreira, P.N, Gutiérrez-Adán, A. and Montoliu, Ll.
A method for the stable introduction of large DNA sequences in the genome of non-human mammals
INIA and CSIC
Spain (OEP: P 200400857) 6 April 2004

ANALISIS FUNCIONAL EL REPRESOR TRANSCRIPCIONAL DREAM

Jose Ramón Naranjo

Summary

La linea de investigación de mi laboratorio durante los últimos años ha ido enfocada a comprender los mecanismod de regulación de la expresión génica en neuronas, en respuesta a señales externas que inducen depolarización de la membrana plasmática. Desde el estudio de la respuesta temprana, en los últimos años nos centramos en el estudio de la señal del calcio y la señalización por estos iones en citosol y/o en núcleo para controlar redes transcripcionales de expresión en poblaciones neuronales concretas frente a estímulos específicos tanto fisiológicos como patológicos.

Nuestros objetivos incluyen i) el conocimiento de estos mecanismos moleculares tanto en sistemas experimentales sencillos como su análisis in vivo mediante modelos animales modificados genéticamente o protocolos usando ARNs de interferencia en animal entero y ii) la caracterización fenotípica de modelos animales de patologías humanas con valor potencial para el rastreo farmacológico o su aplicación en estrategias de terapia génica.

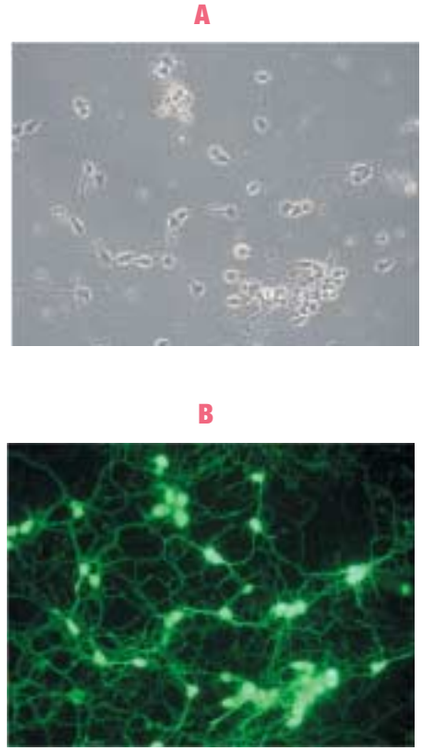

Figura 1. Cultivo de neuronas corticales de ratón expresando la proteína GFP 48 horas despues de la infección con un vector lentiviral codificando para GFP.
A) imagen de contraste de fases.
B) Imagen de fluorescencia mostrando el marcaje GFP.

PERSONNEL

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PUBLICATIONS

Zamorano, A., Lamas, M., Vergara, P., Naranjo, J.R. and Segovia, J. (2003). Transcriptionally mediated gene targeting of gas1 to glioma cells elicits growth arrest and apoptosis. *J. Neurosci. Res.* **71**: 256-63.

Klattenhoff, C., Montecino, M., Soto, X., Guzman, L., Romo, X., De Los Angeles García, M., Mellstrom, B., Naranjo, J.R., Hinrichs, M.V. and Olate, J. (2003). Human brain synembryn interacts with Gsalpha and Gqalpha and is translocated to the plasma membrane in response to isoproterenol and carbachol. *J Cell Physiol.* **195**: 151-157.

Link, A. W., Ledo, F., Torres, B., Palczewska, M., Madsen, T.M., Savignac, M., Albar, J.P., Mellström, B. and Naranjo, J.R. (2004). Day-Night changes in DREAM activity contribute to circadian gene expression in pineal gland. *J. Neurosci.* **24**: 5346-5355.

Rivas, M., Mellström, B., Naranjo, J.R. and Santisteban, P. (2004). Transcriptional repressor DREAM interacts with TTF-1 and Pax-8 and down regulates thyroglobulin gene expression. *J. Biol. Chem.* **279**: 33114-33122.

Zamorano, A., Mellstrom, B., Vergara, P., Naranjo, J.R. and Segovia, J. (2004). Glial-specific retrovirally mediated gas1 gene expression induces glioma cell apoptosis and inhibits tumor growth *in vivo*. *Neurobiol. Dis.* **15**: 483-91.

Mellstrom, B., Torres, B., Link, W.A. and Naranjo, J.R. (2004). The BDNF gene: exemplifying complexity in Ca2+-dependent gene expression. *Crit Rev Neurobio*l **16(1-2)**: 43-50.

RESEARCH PROJECTS

Proyecto 08.5/0045.1/2003 (CAM) Análisis de la regulación de BDNF por mutantes DREAM en cerebro de ratones transgénicos 1/10/2003 - 30/9/2004. 32.619 .

Proyecto GR/SAL/0888/2004 (CAM) Análisis de la funcionalidad de DREAM en la glandula tiroidea. 1/1/2005 - 31/12/2005. 43.960.

Proyecto BMC2001-1431 (CICYT) Análisis de las interaciones proteina-proteina y los genes diana que median los efectos transcripcionales de DREAM. 28/12/2001- 27/12/2004. 207.349.

Proyecto SAF2004-06644 (CICYT) Análisis genómico y fenotípico de modelos transgénicos por sobreexpresión de mutantes del represor DREAM en cerebro. 13/12/2004- 12/12/2007. 293.700.

Proyecto GEN2003-20651-C06-01 (CICYT) Análisis genómico y proteómico de la adicción a psicoestimulantes: papel del represor DREAM. 1/9/2004- 31/8/2007. 253.000.

Project RGP0156/2001-B. (HFSP) Calcium-regulated expression of calcium transporting systems during neuronal development, survival and death (Grant coordinator). June 2001 to May 2004. 187.500 $.

Project LSHM-CT-2004-512039 (VI Framework Program) NoE “Neurone” Molecular mechanisms of neuronal degeneration: from cell biology to the clinic 13/12/2004 - 12/12/2008. 136.850.

MECHANISMS OF INTERACTION BETWEEN THE INFLUENZA VIRUS AND THE INFECTED CELL

Amelia Nieto

Summary

As far as we study the different functions of viral proteins and the mechanisms that viruses utilize to express the genome is becoming more and more important the contribution of the host cell. There are many examples of viruses that divert cellular proteins or RNAs as co-factors for their own transcription and/or replication and many RNA viruses take over the cellular gene expression machinery, leading to the preferential synthesis of viral products and the shut-off of cellular expression.

Influenza virus has a segmented genome of eight RNA chains of negative polarity and its polymerase is composed of three different subunits called PB1, PB2 and PA. For several years we have been involved in: I) the characterization of the individual function of PA polymerase subunit and II) the role of the viral NS1 protein on the specific translational enhancement of viral mRNAs. Along these studies we have looked for cellular factors that could be involved in viral function. These studies forced us to study both the cellular functions of these proteins and their relevance for the virus cycle. As an example of the work done some new undescribed transcription modulators of the RNA polymerase II emerged as necessary for viral expression as PA-interacting proteins. It suggests that viral and cellular polymerases could require common factors for genome expression.

Association of translation initiation factor eIF4GI with NS1 protein was found as involved in the mechanism of specific enhancement of translation of the viral messengers. Then besides to study the aforementioned aspects one biological question appears on top of that: The

influenza virus and the infected cell: for what proteins do they compete and what proteins do they share?

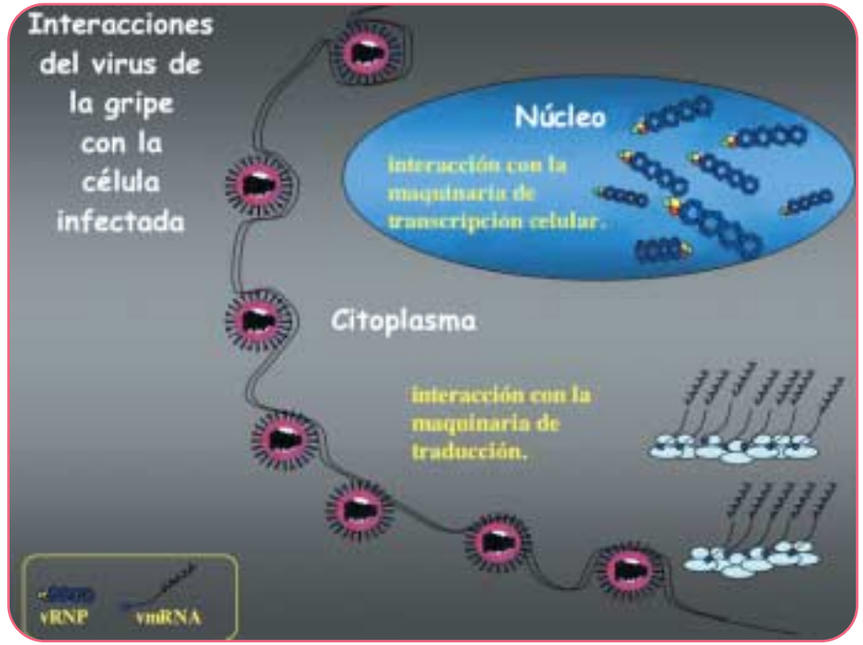

Figure 1. The figure represents the entry of influenza virus in the infected cell. Viral genome is shown in the nucleus of the infected cell, where viral transcription and replication takes place. In yellow it is shown the interactions of the virus with cell transcription and translation.

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PUBLICATIONS

Huarte, M., Falcon, A., Nakaya, Y., Ortin, J., García-Sastre, A. and Nieto, A. (2003). Threonine 157 of influenza virus PA polymerase subunit modulates RNA replication in infectious viruses. *J Virol.* **77**, 6007-6013.

Burgui, I., Aragón, T., Ortín, J. and Nieto, A. (2003). PABP1 and eIF4GI associate to influenza virus NS1 protein in viral mRNA translation initiation complexes. *J. Gen. Virol.* **84,** 3263-3274.

Falcón, A., Marión, R.M., Zürcher, T., Gómez, P., Portela, A., Nieto, A. and Ortín, J. (2004). Defective RNA replication and late gene expression in temperature-sensitive influenza viruses expressing deleted forms of the NS1 protein. *J. Virol.* **78,** 3880-3888.

TRANSCRIPTION AND REPLICATION OF INFLUENZA VIRUS RNA

Juan Ortín

Summary

The Influenza viruses are among the most dangerous human pathogens, since they have a wide animal reservoir and re-emerge continuously in the human population. They constitute a collection of highly variable pathogens that cause respiratory infections in man. Influenza type A viruses contain a segmented genome composed by 8 RNA molecules of negative polarity. Each one replicates and transcribe independently as a ribonucleoprotein (RNP) in the nucleus of infected cells. Our long-term goal is to elucidate the structure of the influenza virus polymerase complex and the RNP to understand the mechanisms by which this molecular machine transcribe and replicate the virus genome. Essential for this aim will also be to unravel the interplay between the virus-encoded RNP and cellular factors involved in these processes, as well as in the post-transcriptional control of virus gene expression. Our group is addressing these objectives by a combination of experimental approaches including structural, molecular and cellular biology techniques.

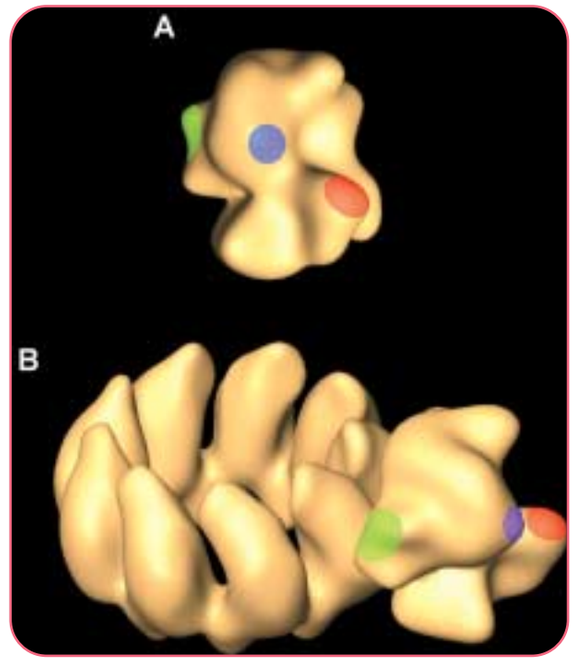

Figure 1. Three-dimensional structure of the influenza virus polymerase (A) and a recombinant mini-ribonucleoprotein with 9 nucleoprotein monomers (B). The coloured areas indicate the aproximate localisation of the polymerase subunits: Red-PB2; Violet-PA; Green-PB1.

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Yolanda Fernández

PUBLICATIONS

Gastaminza, P., Perales, B., Falcón, A.M. and Ortín, J. (2003). Influenza virus mutants in the N-terminal region of PB2 protein are affected in virus RNA replication but not transcription. *J. of Virol.* **77**, 5098-5108

Ortin, J. (2003). Unraveling the replication machine from negative-stranded RNA viruses. *Structure* **11**, 1194-1196

Area, E., Martín-Benito, J., Gastaminza, P., Torreira, E., Valpuesta, J.M., Carrascosa, J.L. and Ortín, J. (2004). Three dimensional structure of the influenza virus polymerase complex: localization of subunit domains. *Proc. Natl. Acad. Sci. USA*, **101**, 308-313.

Falcón, A.M., Marión, R.M., Zürcher, T., Gómez, P., Portela, A., Nieto, A. and Ortín, J. (2004). Defective RNA replication and late gene expression in temperature-sensitive influenza viruses expressing deleted forms of NS1 protein. *J. Virol.* **78**, 3880-3888.

Villacé, P., Marión, R.M. and Ortin, J. (2004). The composition of Staufen-containing RNA granules from human cells indicates their role in the regulated transport and translation of messenger RNAs. *Nucleic Acids Res.* **32**, 2411-2420.

RESEARCH PROJECTS

Red Europea “European Vigilance Network for the Management of Antiviral Drug Resistance” (VIRGIL). Unión Europea FP6-503359. 2004-2007.

Biología Molecular y celular de la replicación del virus de la gripe. Plan Nacional de Investigación Científica y Técnica BFU2004-00491/BMC. 2005-2007.

DOCTORAL THESES

Pablo Gastaminza Landart (2003).
La subunidad PB2 de la polimerasa del virus de la gripe es esencial para la replicación viral.
Universidad Autónoma de Madrid.

Ana M. Falcón Escalona (2003).
La proteína NS1 del virus de la gripe: Actividades nucleares y citosólicas e implicaciones en la patogénesis viral.
Universidad Autónoma de Madrid.

Estela Area Gómez (2004).
Modelos tridimensionales de la ribonucleoproteína y la polimerasa del virus de la gripe.
Universidad Autónoma de Madrid.

Patricia Villacé Lozano (2004).
Caracterización de los complejos ribonucleoproteicos que contienten la proteína humana Staufen. Implicación en el transporte y traducción localizada de RNAs mensajeros.
Universidad Autónoma de Madrid.

MOLECULAR CHARACTERIZATION OF TOROVIRUSES

Dolores Rodríguez Aguirre

Summary

Toroviruses are enveloped viruses, with a positive single stranded RNA genome, and highly pleomophic viral particles.

These viruses were identified for the first time in 1972, and they have been recently included as a new genus within the *Coronaviridae* family.

The scarce epidemiological studies performed in different countries indicate that toroviruses may be an important cause of gastroenteritis both in humans and in different animal species of major importance in the livestock. Despite this fact, their large geographical distribution, and their ability to infect a broad variety of animal species, these viruses have been poorly characterized.

The main factor that has hampered their study is the impossibility of growing toroviruses in cultured cells, except the equine isolate (BEV), that was the first torovirus been identified.

To start this project the first objective of our laboratory was the development of tools that would let us to undertake the study of the molecular biology of torovirus, and to establish diagnostic procedures with which we will be able to determine their incidence in the human population as well as in the livestock industry.

For this, we used heterologous expression systems (baculovirus and vaccinia virus recombinants) for the expression of the BEV structural proteins. With these means we can study the protein properties in the absence of BEV infection, and to purify these proteins to produce specific polyclonal and monoclonal antibodies. With these antibodies we will be able to follow viral structural proteins during the morphogenetic pathway by confocal and immunoelectron microscopy. The purified proteins will be also used as antigen for the detection of sera positive against torovirus.

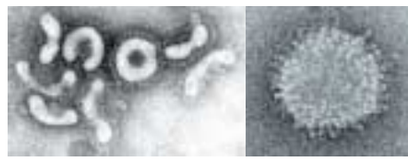

Figure 1. Purified particles of the equine torovirus BEV observed by electron microscopy.

PERSONNEL

Group Leader:
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Ana Mª Maestre Meréns (since Marzo 2003)
Jaime Pignatelli Garrigós (since Sept. 2003)

PUBLICATIONS

Ramiro, M.J., Zarate, J.J., Hanke, T., Rodríguez, D., Rodríguez, J.R., Esteban, M., Lucientes, J., Castillo, J.A. and Larraga, V. (2003). Protection in dogs against visceral leishmaniasis caused by *Leishmania infantum* is achieved by immunization with a heterologous prime-boost regime using DNA and vaccinia recombinant vectors expressing LACK. *Vaccine* **21**, 2474-2484.

González-Aseguinolaza, G., Nakaya, Y., Molano, A., Dy, E., Esteban, M., Rodríguez, D., Rodríguez, J.R., Palese, P. and García-Sastre, A., Nussensweig. (2003). Induction of protective immunity against malaria by priming-boosting immunization with recombinant cold-adapted influenza and modified vaccinia Ankara viruses expressing a CD8+-T-cell epitope derived from the circunsporozoite protein of *Plasmodium yoelii*. *J. Virol.* **77**, 11859-11866.

Gallego, J.C., Risco, C., Rodríguez, D., Cabezas, P., Guerra, S., Carrascosa, J.L. and Esteban, M. 2003. Differences in virus-induced cell morphology and in virus maturation between MVA and other strains (WR, Ankara and NYCBH) of vaccinia virus in infected human cells. *J. Virol.* **77**, 10606-10622.

Gómez, C.E., Abaitua, F., Rodríguez, D. and Esteban, M. (2004). Efficient CD8+ T cell response to HIV-env V3 loop epitope from multiple isolates by a DNA prime/vaccinia virus boost (rWR and rMVA strains) immunization regime and enhancement by the cytokine IFN-γ. *Virus Research* **105**, 11-22.

RESEARCH PROJECTS

Dolores Rodríguez-Aguirre.
Vectores multiepitópicos como vacuna contra malaria.
Ministerio de Ciencia y Tecnología. CICYT (BIO 99-0803). 47.118 €, 2000-2003.

Dolores Rodríguez-Aguirre.
Morfogénesis del virus vaccinia: estudios bioquímicos e inmunocitoquímicos.
Comunidad Autónoma de Madrid (CAM 082.2/0042.1/2000). 25.224 €, 2001- 2002.

Dolores Rodríguez-Aguirre.
Torovirus humano: caracterización genética y funcional. Desarrollo de herramientas para su diagnóstico en clínica.
Ministerio de Ciencia y Tecnología. CICYT (BIO 2002-03739), 2003-2006. 117.300 €.

PATENTS

Rodríguez Aguirre, J.F., Ruiz Castón, J., González Llano, M.D., Rodríguez Aguirre, M.D., Blanco Chapinal, S., Oña Blanco, A., Salgar Gómez, I., Abaitua Elustondo, F., Luque Buzo, D. and Rodríguez Fernández-Alba, J.R.
Cápsidas vacías quiméricas del virus causante de la enfermedad de la bursitis infecciosa (IBDV), su procedimeinto de obtención y aplicaciones/ Chimeric empty capsids from the virus causing the infectious bursal disease (IBDV), obtention procedure and applicatons.
CSIC y BIONOSTTRA S.L.
Nº de solicitud 200400120. Ampliación a patente internacional PCT/EP2005/000695

MOLECULAR BIOLOGY OF BIRNAVIRUS

José F. Rodríguez-Aguirre

Summary

Members of the Birnaviridae family are characterized by possessing a bipartite dsRNA genome enclosed within an icosahedral capsid formed by a single protein layer.

Some birnaviruses have a major economic impact on the poultry and fish-farming industries.

Current birnavirus control measures, mainly based on live vaccines, are rather inefficient. A good example of this is the situation of Infectious bursal disease virus (IBDV), our major working model.

The systematic use of live IBDV vaccines has not prevented the spread of the disease and the constant increase on virus virulence. Our goal is to develop efficient and safe strategies to controlling birnavirus-borne diseases.

We believe that the only realistic way to achieve this goal is by getting a deep understanding of key aspects of the birnavirus molecular biology.

We have focussed our effort on three main topics: i) IBDV morphogenesis and structure; ii) Development of IBDV subunit vaccines; and iii) Generation of genetically-resistant chicken lines.

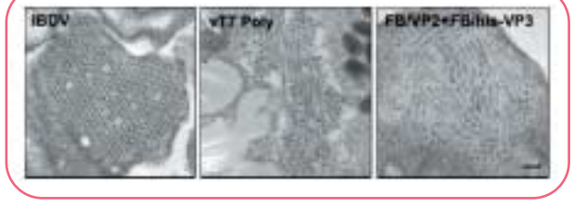

Figure 1. Detection of IBDV assemblages formed in different expression systems.EM images correspond tochicken embryo fibroblasts infected with IBDV, BSC-1cells infected the recombinant vaccinia virus vT7 Poly that expresses de IBDVpolyprotein, and H5 insect cells coinfected with the recombinant baculoviruses FB/pVP2 and FB/his-VP3, respectively. Scale bar indicates 250 nm

PERSONNEL

Group Leader:
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Mª Dolores González de Llano, científico titular OPIS

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PUBLICATIONS

Maraver, A., Clemente, R., Rodríguez, J.F., Lombardo, E. (2003). Identification and molecular characterization of the RNA polymerase-binding motif of infectious bursal disease virus inner capsid. *J. Virol.* **77**, 2459-2468.

Kochan, G., González, D. and Rodríguez, J.F. (2003). Characterization of the RNA-binding activity of VP3, a major structural protein of Infectious bursal disease virus. *Arch. Virol.* **148**, 723-744.

Maraver, A., Oña, A., Abaitua, F., González, D., Clemente, R., Ruiz-Diaz, J.A., Caston, J.R., Pazos, F. and Rodríguez, J.F. (2003). The oligomerization domain of VP3, the scaffolding protein of infectious bursal disease virus, plays a critical role in capsid assembly. *J. Virol.* **77**, 6438-6449.

Cattoli, G., Terregino, C., Brasola, V., Rodríguez, J.F. and Capua, I. (2003). Development and preliminary validation of an ad hoc N1-N3 discriminatory test for the control of avian influenza in Italy. *Avian Dis.* **47**: 1060-1062.

Capua, I., Terregino, C., Cattoli, G., Mutinelli, F. and Rodríguez, J.F. (2003). Development of a DIVA (Differentiating Infected from Vaccinated Animals) strategy using a vaccine containing a heterologous neuraminidase for the control of avian influenza. *Avian Pathol.* **32**: 47-55.

Eichwald, C., Rodríguez, J.F. and Burrone, O. (2004). Characterisation of rotavirus NSP2/NSP5 interaction and dynamics of viroplasm formation. *J. Gen. Virol.* **85,** 625-634.

Oña, A., Luque, D., Abaitua, F., Maraver, A., Castón, J.R. and Rodríguez, J.F. (2004). The C-terminal domain of the pVP2 precursor is essential for the interaction between VP2 and VP3, the capsid polypeptides of infectious bursal disease virus. *Virology* **322,** 135-142.

RESEARCH PROJECTS

José F. Rodríguez-Aguirre.
Phylogenetic sequence analysis and improved diagnostic assay systems for viruses of the family *Reoviridae*
EU, 199,000, 01-01/06-04.

José F. Rodríguez-Aguirre.
IBDV morphogenesis
Ministerio de Ciencia y Tecnología, Spain, 133,250, 01-01/12-03.

José F. Rodríguez-Aguirre.
Development of IBDV oral vaccines
Comunidad Autónoma de Madrid, Spain, 108.182 , 01-01/12-02.
José F. Rodríguez-Aguirre.
Development of IBDV subunit vaccines
Comunidad Autónoma de Madrid, Spain, 130,000 , 06-03/06-05.

José F. Rodríguez-Aguirre.
Development of IBDV marker vaccines
Ministerio de Ciencia y Tecnología, Spain, 203,500 , 01/12/03-30/11/06.

DOCTORAL THESES

Roberto Clemente Cervera (2004).
Estudio del papel funcional de la proteína estructural VP3 en el proceso morfogenético del virus de la bursitis infecciosa.
Universidad Autónoma de Madrid, Facultad de Ciencias Biológicas.

PATENTS

Rodríguez, J.F., González de Llano, D., Oña, A., Abaitua, F., Maraver, A., Clemente, R., Castón, J.R. and Rodríguez, J.R.
Procedimiento de producción de partículas vacías (VLPS) del virus inductor de la bursitis infecciosa (IBDV), composiciones necesarias para su puesta a punto y su uso en la elaboración de vacunas frente al IBDV.
CSIC/ BIONOSTRA S.A.
Patent#: 200300751, date: 31.03.03.

Rodríguez Aguirre, J.F., Ruíz Castón, J., González de Llano, M.D., Rodríguez Aguirre, M.D., Blanco Chapinal, S., Oña Blanco, A.M., Saugar Gómez, I., Abaitua Elustondo, F., Luque Buzo, D., y Rodríguez Fernández-Alba, J.R.
Cápsidas vacías quiméricas del virus causante de la enfermedad de la bursitis infecciosa (IBDV), su procedimiento de obtención y aplicaciones.
CSIC/ BIONOSTRA S.A
Patent#: P200400120, date: 21.01.2004.

Rodríguez, J.F., Ruíz Castón, J., González de Llano, M.D., Oña Blanco, A.M., Abaitua Elustondo, F., Luque Buzo, D. y Rodríguez Fernández-Alba, J.R.
Cápsidas vacías (VLPs(-VLP4)) del virus causante de la enfermedad de la bursitis infecciosa (IBDV) su procedimiento de obtención y aplicaciones.
CSIC/ BIONOSTRA S.A
Patent#: P200400121, date: 21.01.2004

Ruíz Castón, J., Saugar Gómez, I., Luque Buzo, D., Abaitua Elustondo, F., Oña Blanco, A.M., González de Llano, M.D., Rodríguez Aguirre, J.F. y Rodríguez Fernández-Alba, J.R.
Title: Procedimiento para la producción en levaduras de cápsidas virales vacías compuestas por proteínas derivadas de pVP2 del virus causante de la enfermedad de la bursitis infecciosa (IBDV).
CSIC/ BIONOSTRA S.A.
Patent#: P20041044, date: 30.04.04

FORMACIÓN Y FUNCIÓN DE LOS mRNAS

Carlos Mª Suñé Negre

Summary

Expression of protein-coding genes is a multi-step process beginning with transcription by RNA polymerase II (RNAPII) in the nucleus. During transcription, the nascent pre-mRNA undergoes several processing steps including capping, splicing, and polyadenylation.

The mature mRNA is then exported to the cytoplasm for translation. A distinct cellular machine carries each of the steps in gene expression out, but growing evidence indicates that there is an extensive network of coupled interactions between each machine.

Although the existence of these connections is widely accepted, their nature remains to be elucidated. The long-term goal of our laboratory is to understand the physical and functional connections between the mRNA transcription elongation and processing.

Part of the laboratory is studying a protein that may hold a key to a subset of these connections, the transcription elongation factor CA150. The other part broadens the scope of the laboratory project by studying the molecular mechanisms of transcription elongation by RNAPII.

Current projects in the lab involve the following aims:

i) Functional and biochemical characterization of the connections between CA150 and the splicing machinery.

ii) Molecular analysis of RNAPII transcription complexes.

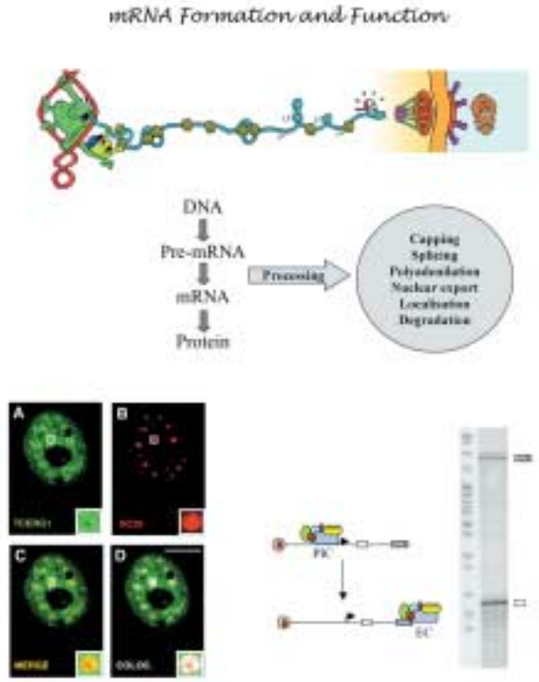

Figure 1. A high body of evidence demonstrates the importance of transcriptional control in regulating gene expression. The long-term goal of our laboratory is to understand the physical and functional connections between the mRNA transcription elongation and processing. Part of the laboratory is studying a protein that may hold a key to a subset of these connections, the transcription elongation factor TCERG1/CA150 (confocal microscopy image, left panel: TCERG1/CA150 colocalizes with splicing factors). The other part broadens the scope of the laboratory project by studying the molecular mechanisms of transcription elongation by RNAPII (scheme and gel image, right panel: isolation and functional characterization of pre-initiation [PIC] and elongation [EC] transcription complexes)

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PUBLICATIONS

Suñé, C., Brennan, L.E., Stover, D.R., and Klimkait, T. (2004). Effect of polymorphisms on the replicative capacity of protease inhibitor-resistant HIV-1 variants under drug pressure. Clin. *Microbiol. Infect.* **10**, 119-126.

RESEARCH PROJECTS

Carlos María Suñé Negre

Control transcripcional de la expresión génica del VIH-1: papel del coactivador transcripcional CA150 (SAF 2002-02641) Ministerio de Ciencia y Tecnología, entidades participantes: Centro Nacional de Biotecnología. Madrid. España, desde: 03/2003 hasta: 03/2006.

VIRAL MODULATION OF THE IMMUNE RESPONSE

Antonio Alcamí
(adscripción temporal desde marzo 2004)

Summary

The main objective of our laboratory is the understanding of the interaction of large DNA viruses (poxviruses and herpesviruses) with the host immune system. The battle between viruses and the immune system has been going on for millions of years of virus-host co-evolution. Our hypothesis is that viruses have influenced the evolution of various aspects of immunity and viral genomes can be considered repositories of information on the host immune system. A better understanding of viral immune evasion strategies will provide information on viral pathogenesis, the function of the immune system and new strategies of immune modulation with therapeutic applications. Our research can be divided into several areas: (1) identification and characterization of novel poxvirus and herpesvirus proteins that mimic host cytokine receptors and inhibit the activity of cytokines and chemokines; (2) investigations on mousepox, a disease caused by ectromelia virus, as a natural mouse model of infection to understand the contribution of viral proteins to pathogenesis and immune modulation; and (3) the development of viral immune modulatory proteins as anti-inflammatory reagents that may be used in the clinic to treat human diseases caused by immunopathological reactions.

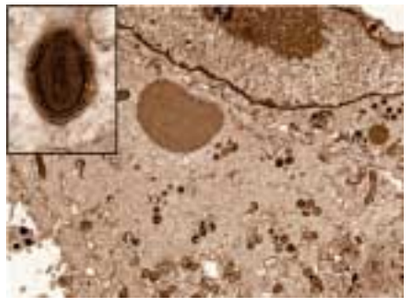

Figure 1. Electron micrographs of cells infected with ectromelia virus.

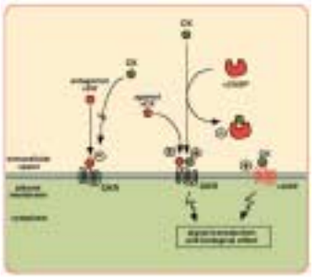

Figure 2. Viral modulation of chemokine activity: viral chemokine binding proteins (vCKBP) and molecular mimicry of chemokines (vCK) and chemokine receptors (vCKR).

PERSONNEL

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PUBLICATIONS

Bryant, N.A., Davis-Poynter, N., Vanderplasschen, A. and Alcami, A. (2003). Glycoprotein G isoforms from some alphaherpesviruses function as broad-spectrum chemokine binding proteins. *EMBO J.* **22**, 833-846.

Ribas, G., Rivera, J., Saraiva, M., Campbell, R.D. and Alcami, A. (2003). Genetic variability of immunomodulatory genes in ectromelia virus isolates detected by denaturing high performance liquid chromatography. *J. Virol.* **77**, 10139-10146.

Alcami, A. (2003). Viral mimicry of cytokines, chemokines and their receptors. *Nature Rev. Immunol.* **3**, 36-50.

Webb, L.M.C., Smith, V.P. and Alcami, A. (2004). The gammaherpesvirus chemokine binding protein can inhibit the interaction of chemokines with glycosaminoglycans. *FASEB J.* **18**, 571-573.

Pyo, R., Jensen, K.K., Wiekowski, M.T., Manfra, D., Alcami, A., Taubman, M.B. and Lira, S.A. (2004). Inhibition of intimal hyperplasia in transgenic mice coditionally expressing the chemokine binding protein M3. *Am. J. Pathol.* **164**, 2289-2297.