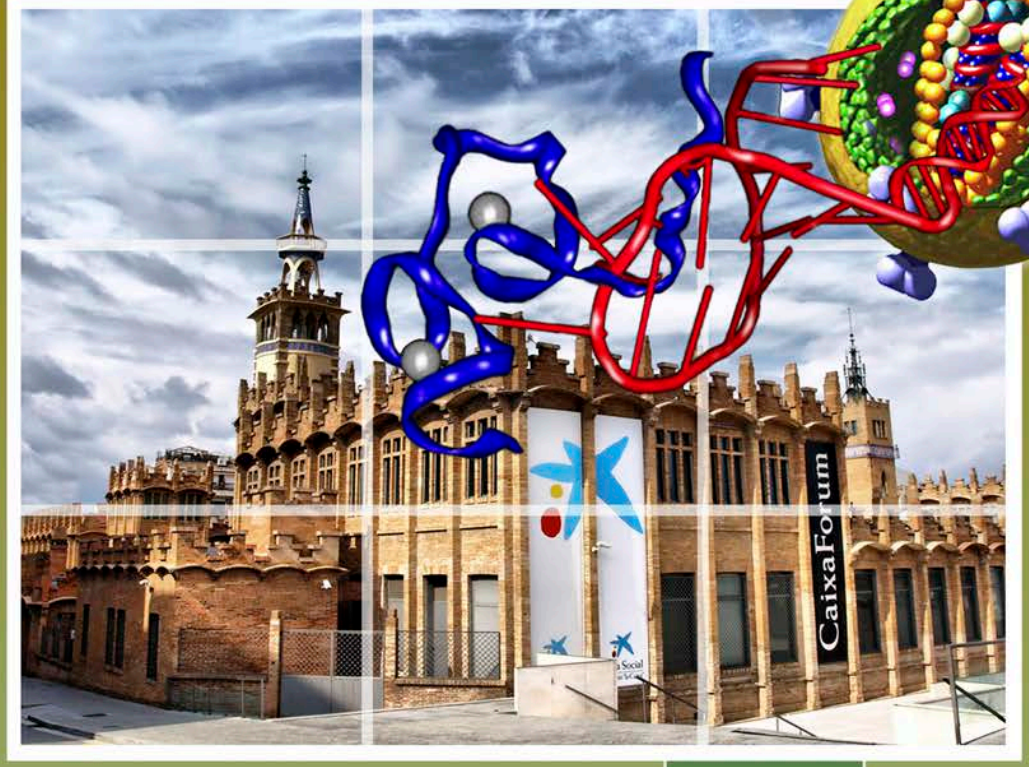
8th International Retroviral NC Symposium



September 18-21, 2011
CaixaForum
Barcelona • Spain

biocat International Center for Scientific Debate

Initiative fostered by: Welfare Projects "la Caixa" Foundation

Eighth International Retroviral NC Symposium

September 18-21, 2011

CaixaForum de Barcelona

Av. de Francesc Ferrer i Guàrdia, 6-8 08038 Barcelona, Spain

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Introduction

After 14 years of intensive research, especially that dedicated to HIV, the purpose of this 8th International Retroviral NC Symposium is to exchange the latest results and ideas related to retroviral Nucleocapsid (NC) Proteins and functionally homologous proteins in other viruses.

NC proteins are central to retroviral replication. They are vital for the retroviral RNA-DNA choreography with their paradigmatic nucleic acid chaperone function supported by their highly conserved zinc fingers. Moreover, their interactive and architectural properties support the retroviral plasticity required for cell-to-cell spread, from virion assembly and budding through nuclear entry of the completed viral DNA. Retroviral NC proteins are processed from their Gag precursors by the viral protease, whereupon they directly assist reverse transcriptase in viral DNA synthesis. With the integration of modern biology and powerful biophysical methods, impressive progress is expected for a more complete understanding of NC.

As their function is obligatory for retroviral replication at many steps, NC is a prime target for HIV, as well as retroviruses involved in cancer such as HTLV-1 or human MLV-like (XMRV) retroviruses. Additionally, functionally homologous proteins from other viruses such as HCV and SARS-coronavirus are similarly under investigation and will be discussed.

The topics to be presented will cover up-to-date concepts of modern biology, supported by the latest applications of physics and biotechnologies, in particular new microscopic methods. A short session dedicated to HIV-1 NC-related translational research will end the symposium.

Invited speakers have been selected for their outstanding contributions in the NC field. We are also honored by the presence of Françoise Barré-Sinoussi, who will open the Symposium, focusing on the persistent challenge to target HIV-1 key players, and of Hans-Georg Kräusslich, who will direct us to a detailed view of HIV-1 maturation.

Held at the contemporary art museum CaixaForum of Barcelona, the venue will stimulate discussions on new avenues for the study and exploitation of these proteins as targets for antiviral therapies. Inspired by this location, the 8th IRNCS will be also honored by the presence of Louis Henderson. His artwork dedicated to illustrate HIV, as well as HSV, will be displayed during the symposium.

The organizers are grateful to the following sponsors for providing financial support for the Symposium.

Key Supports from Barcelona

http://obrasocial.lacaixa.es

From its centres in Barcelona, Palma, Tarragona, Lleida and Madrid, **CaixaForum** has been designed by **La Caixa Foundation** as a space for dissemination and debate on culture and its many manifestations. It offers a miscellaneous range of subjects that covers all periods, from the initial manifestations of art to the most innovative and recent avant-garde, and feature the greatest ever exponents of art and culture. CaixaForum also periodically runs cycles of conferences, seminars, courses, concerts, performances and special events that help to prompt reflection on this changing world and better understanding both of our society and that of other cultures. Activities are designed for all audiences and entrance is free, bringing art and culture to a wider audience. La Caixa Foundation also offers meeting spaces for exchanging ideas about what is happening at the time in the scientific community, on scientists' recent innovations and on their imminent achievements. La Caixa Foundation works together with universities, public and private research centres and university hospitals to generate new scientific knowledge. In its eagerness to fight the most serious illnesses affecting the public and to promote medical research in different fields, its collaborative work includes the notable association with the Irsicaixa Fondation, a benchmark institute in the fight against AIDS. It funds HIVACAT, the HIV vaccine research and development program developed in Catalunya.

http://www.biocat.cat/en/icsd

The **International Center for Scientific Debate (ISCD)** is an initiative of Biocat, fostered by the Welfare Projects "la Caixa", which aims to drive top-notch international scientific meetings to promote debate and open exchange of knowledge among national and international experts of renowned prestige. The meetings are global, integrative and multidisciplinary focused, aiming to tackle social needs in the field of life sciences. Taking into consideration the complexity and constantly changing conditions of the World. The ISCD also collaborates in the dissemination of knowledge, approaching science to society and contributing to position Barcelona as a city of knowledge and Catalonia as a country of scientific excellence.

http://www.hivacat.org/

HIVACAT is the Catalan project for the development of an effective vaccine against the HIV virus. The organization is a joint public and private sector consortium unprecedented in Spain, placing the country at the forefront of international research in this field. Composed of Catalonia's two longest established and leading AIDS research centers, IDIBAPS and the IrsiCaixa Institute for AIDS Research, HIVACAT conducts research into the development of a new HIV vaccine, in conjunction with the organization ESTEVE, and with the support of the La Caixa Foundation, the autonomous Catalan government's Department of Health and Department of Innovation, Universities and Companies plus the Clinic Foundation at Barcelona's Hospital Clinic. This consortium is the first major collaboration attempt between local government, research centers and private enterprise in this field.

Historical Mainstays

The **Office of AIDS Research** (**OAR)** coordinates the scientific, budgetary, legislative, and policy elements of the NIH AIDS research program. Through its annual comprehensive trans-NIH planning, budgeting, and portfolio assessment processes, OAR sets scientific priorities, enhances collaboration, and ensures that research dollars are invested in the highest priority areas of scientific opportunity that will lead to new tools in the global fight against AIDS.

http://www.oar.nih.gov/

Created in 1992 and financed by the French government, the **ANRS** (**Agence nationale de Recherches sur le SIDA**) coordinates and funds research on AIDS and viral hepatitis B and C in France and developing nations. The primary objective of the ANRS is to define scientific priorities and mobilize the scientific community, as well as to finance programs and research grants after evaluation by scientific authorities.

http://www.anrs.fr/

SAIC-Frederick, a wholly owned subsidiary of SAIC, is speeding the delivery of new technologies and treatments to patients with cancer and AIDS. We operate the laboratories at the National Cancer Institute at Frederick (NCI-Frederick) under the largest contract awarded by the U.S. Department of Health and Human Services.

http://www.saic-frederick.com/

Catalan/Spanish Supports

The **Ramón Areces Foundation** is a privately-owned, non-profit institution created in 1976 with the wide-ranging aim of encouraging scientific and technical research in Spain, as well as education and culture in general - values that the institution considers to be fundamental drivers for social progress and modernity. The institution carries out its activities throughout Spain in the areas of the Materials and Life Sciences, the Social Sciences and the Humanities - areas in which it fosters scientific research, contributes to the training of human capital and disseminates knowledge. For each of these areas, the institution has put in place its own programmes: National Competitions for Research Funding, Scholarships for Study Abroad and Programmes for Scientific, Socio-Economic and Cultural Dissemination. Furthermore, the Ramón Areces Foundation acts in these fields by providing support to institutions of great prestige such as the Royal Academies, the National Centre for Cardiovascular Research, the Severo Ochoa Centre for Molecular Biology and the Albéniz Foundation.

www.fundacionareces.es

http://www.retic-ris.net

RIS is the Spanish Network for AIDS research. It Is supported by the Spanish Ministry of Science and Innovation (MICIN) in the context of the RETICS. It was created in 2004 and contains three research platforms (CoRIS, Biobank and Resistence) and four research lines (epidemiology, vaccines, immunopathogenesis and strategic studies including clinical trials). It is composed by near 30 research groups (basic science, clinical science and epidemiology) and has greatly contributed to the Spanish research in the field of HIV/AIDS during past few years including international collaborations and visualization.

www.IDIBAPS.org

The Institut D'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) is a research center formed by the Generalitat de Catalunya's Ministry of Innovation, Universities and Enterprise; the University of Barcelona; the Hospital Clínic of Barcelona and the Institut D'Investigacions Biomèdiques de Barcelona of the Council for Scientific Research (IIBB-CSIC). IDIBAPS's fundamental objective is to integrate quality clinical research with high level translational and basic research.

Supporters of the NC travel grant

http://www.sidaction.org/

Sidaction, a France-based NGO, is a diverse coalition of individuals and organizations from France and from developing countries. Dedicated to fundraising, advocacy, and technical assistance to fight HIV/AIDS in France and in 29 low and middle income countries, Sidaction raises private funds to promote cutting-edge scientific research and to improve access to prevention, care, treatment, and support programs.

The **Fundació Lluita contra la sida** is comprised of doctors, psychologists, dieticians, nurses, social workers and other professionals to offer multidisciplinary and quality healthcare to people with HIV. The research activities help many patients to participate in clinical trials with new drugs. The Fundació Lluita contra la sida was the embryo of the retrovirology laboratory that eventually became the Fundació IrsiCaixa. As years have passed, the Foundation's role has consolidated and it has become a key element in the HIV working group at the Hospital. Its work has gradually come to focus on three major areas: Patient care – Research – Teaching.

http://www.rnasociety.org/

The **RNA Society** was formed in 1993 to facilitate sharing and dissemination of experimental results and emerging concepts in ribonucleic acid research. The Society encompasses RNA research in the broadest sense: from the ribosome to the spliceosome, from RNA viruses to catalytic RNAs. It is a multidisciplinary society, representing molecular, evolutionary and structural biology, biochemistry, biomedical sciences, chemistry, genetics, and virology as they relate to questions of the structure and function of RNA and of ribonucleoprotein assemblies. The RNA journal is a publication of the RNA Society.

Last minute Supporters: Biotech companies from South Europe

http://www.synprosis.com

SYNPROSIS is specialized in the design and the synthesis of peptides and small proteins for research needs or GMP. Synprosis' expertise enables production of "long and / or complex" peptides: folded polypeptides up to 200mer, with natural or modified amino acids, like defensins, toxins, etc…Synprosis is particularly active in the development of APIs for vaccines and offers various possibilities for building antigenic peptides (long linear peptides, dendrimers (MAP), conjugation to different carrier proteins, etc.).
To facilitate and expedite the development of your projects, Synprosis proposes a "Fast track peptide drug development" protocol: API (from design, analytical development to the GMP production), drug product (from formulation studies to the stability studies), provision of first batch for clinical trial, technical and regulatory support.

http://www.diatheva.com/

DIATHEVA is a biotechnological company focused on development, production and marketing of new and innovative products for diagnostics, research and therapeutic applications in the filed of: Poverty Related Diseases, Cancer and Pharmacogenetics. DIATHEVA has a GMP authorized facility designed for the manufacture of APIs for pre-clinical and clinical trial studies with a special emphasis on new immunogens against HIV and other microbial pathogens.

Program

Sunday - September 18, 2011

CaixaForum

15:30-17:30 PM **Registration / Check-in at Catalonia Plaza Hotel**

18:00 PM Opening Remarks by Gilles Mirambeau, José Maria Gatell and Aloha Congress
Welcome, the 8th IRNCS, Posters and other Logistics, Sponsors

18:20 PM **Keynote address** 1
Françoise Barré-Sinoussi*, France
Past successes and future challenges in targeting key players of HIV pathogenesis

19:20 PM **Extra session about the Mediterranean diet**
Emilio Ros*, Spain
My Grandma was right

20:00 PM *Cocktail / Dinner at Museu d'Art de Catalunya* (http://www.mnac.cat)

*Invited speakers # Selected talk

Monday - September 19, 2011

CaixaForum

SESSION 1: 9:00 AM - 13:10 PM

From Translation to Assembly

Chairpersons: Drs. Delphine Muriaux and Andrew Mouland

9:00 AM **Suzanne Sandmeyer*, USA,** 2
Functions of the Ty3 retrotransposon NC protein
lecture

9:30 AM **Theophile Ohlmann*, France** 3
Translation and localization of HIV genomic RNAs

Tuesday - September 20, 2011

CaixaForum

8:45 AM **SESSION 2 : 8:45 – 10:55 AM**

Interaction with cellular factors

Chairperson: Dr. Lawrence Kleiman

8:45 AM **Eric Freed[*], USA** 13

Host cell factors in retrovirus assembly and release

Lecture

9:15 AM **Kuan-Teh Jeang[*], USA** 14

Cellular factors that contribute to HIV-1 post-transcriptional gene expression

9:35 AM **Yeng Hui Zheng[*], USA** 15

MOV10 antiretroviral mechanism

9:55 AM **Lawrence Kleiman[*], Canada** 16

Evidence that Gag promotes the cytoplasmic annealing of $tRNA^{LYS3}$ to HIV-1 viral RNA

10:15 AM **Gaelle Mercenne[#], USA** 17

Identification and characterization of Gag-binding cellular cofactors required for HIV-1 replication

10:35 AM **Fadila Bouamr[#], USA** 18

The role of NC in virus budding

10:55 AM- *Coffee break*

SESSION 3 : 11:15 AM – 15:15 PM

The biology and physical chemistry of RNA chaperones

***Chairpersons: Drs. Karin* Musier-Forsyth and Luis Enjuanes**

11:15 AM **Jean-Luc Darlix[*], France** 19

Serendipity behind the search for Retroviral Nucleocapsid structures and functions

Lecture

16:10 PM **Gilles Mirambeau[*], Spain & France** 29

Towards a mechanistic insight of HIV-1 NC maturation

16:30 PM **Jun-ichi Sakuragi[#], Japan** 30

A proposal of new structural model of HIV-1 DLS

16:50 PM **Robert Gorelick[*], USA** 31

Premature reverse transcription is not the cause of the replication defect in NC-mutant HIV-1

17:10 PM **Christopher P. Jones[#], USA** 32

Dissecting the role of HIV Gag's zinc fingers in nucleic acid binding, chaperone activity and premature reverse transcription

17:30 – 19:30 PM **Poster session 2 and Louis Henderson's Artwork session**
CaixaForum Aula Laboratory

20:30 PM Dinner at Catalonia Plaza Hotel

22:00 PM Live Jazz at the plaza piano bar Show

Wednesday – September 21, 2011
CaixaForum

9:00 AM **Special keynote**

Hans-Georg Kräusslich[*], Germany

Proteolytic maturation of the HIV-1 core

SESSION 5 : 10.00 AM – 15:40 PM

Resonance, stretching, shaping, tapping & HiRes imaging

Chairpersons : Drs. Carine Tisné and Mark Williams (morning)

Drs Louis Mansky and Sébastien Lyonnais (afternoon)

10:00 AM **Michael Summers[*], USA** 33

Structural insights into the mechanism of HIV-1 genome selection and packaging

SESSION 6 : 16:00 – 17:10 PM

HIV translational research

Chairperson : Dr. Jeffrey Lifson

NC travel grants

NC travel grants have been awarded to:

Karolina Bohmova, Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic
Kristina Clemens, University of California, Irvine, USA
Katherine Fenstermacher, University of Maryland, College Park, USA
Abhijit Jadhav, NY State University, Albany, USA
Mette Jepsen, iNano Center, Aarhus University, Denmark
Zhefeng Meng, Shanghai Public Health Clinical Center, Shangai, China
Mithun Mitra, National Institute of Health, Bethesda, USA

With a special **RNA Society** award to:

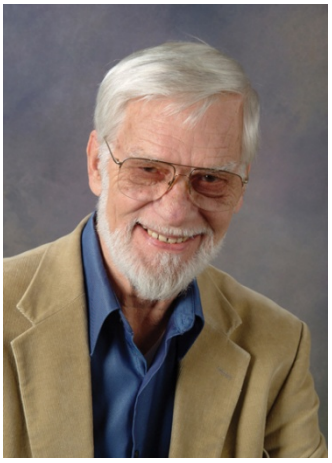
James Munro, Yale University, Newhaven, USA

With a special **SIDACTION** award to:

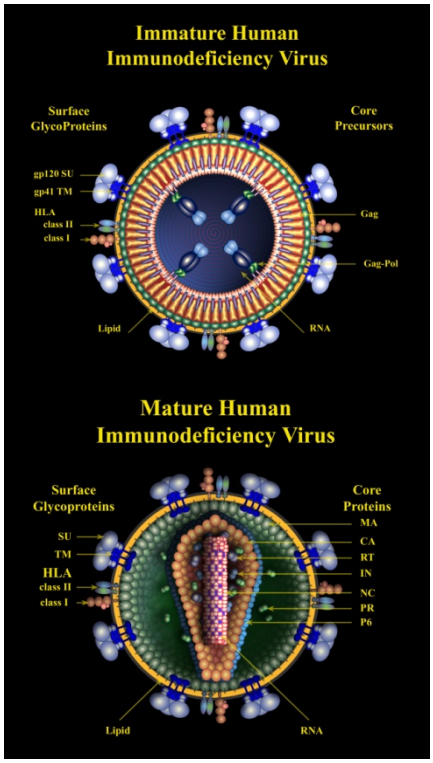
Suriya Aktar, Faculty of Medicine & Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates
Ali Bazzi, LBPA ENS Cachan, France
Laure Dufau, UMPC – Sorbonne Universités, Paris, France
Anne L'Hernault, University of Cambridge, Cambridge, UK
Gaelle Mercene, University of Utah, Salt Lake City, USA
Arunava Roy, University of Calcutta, Calcutta, India

With a special **FLS** award to:

Matia Mori, Universita di Siena, Siena, Italy
Kathy Chaurasiya, Northeastern University, Boston, USA



At this symposium, we will be displaying the scientific artwork of Dr. Louis Henderson who has retired from NCI-Frederick. Lou initiated his professional career at the University of Colorado in the early '60s where he got his start in protein chemistry, enzymology and chromatographic separation of proteins, laying the foundation for the seminal later work for which he is best known. He then traveled east to Harvard University as a Postdoctoral Fellow, then to the University of Goteborg, Sweden then back to Yale University in the United States as a Research Associate where he studied carbonic anhydrase, honing his protein sequencing skills. In 1976, he joined the staff at NCI-Frederick, where he pioneered the use of reverse phase HPLC methods for the separation of viral proteins. Despite always being more interested in working on the next experiment than on a new manuscript, over his career he authored or coauthored over 120 publications, on topics ranging from isotope exchange and enzyme activity of lysozyme and trypsin, to characterization of carbonic anhydrase, retroviral protein composition, purification and characterization of IL-2, retroviral inhibitors targeting the NC protein, cellular proteins associated with retroviruses, and many other subjects.



Later in his career, Lou became very interested in exploring the use computer-based digital drawing programs to replace the tedious traditional methods for preparing graphics for scientific publications and slide presentations. Initially, he surveyed several digital drawing programs that were available for the PC and selected Macromedia's Freehand program as the then available program best suited for the job. He started preparing two-dimensional (2-D) objects and figures in color and through his efforts convinced the local Graphics Arts department at NCI-Frederick to start generating graphics by computer. One of the earliest figures that Lou created with Freehand took on a life of its own. The figure is a cartoon sketch showing cross-sectional views of an immature and mature retrovirus. The NIH AIDS Research and Reference Reagent Program had the figure made into posters that were distributed at various conferences, which wound up being displayed on laboratory walls all over the world and was later used in some textbooks. It was also incorporated into an art exhibition entitled "Art in Science" that was held in New York in 2001.

Dr. Henderson has refined his digital graphics methods, making use of primarily two programs, Adobe Photoshop and Macromedia Extreme 3-D used to generate object and lighting effects that create the illusion of three dimensions. Most of the images displayed at this exhibition were prepared using these programs. His images have been used on journal covers, in innumerable scientific presentations, and have also been displayed in gallery shows. The familiar saying, "A picture is worth a thousand words," was coined by Fred R. Bernard in an article published in the Printers' Ink, 1921 (Wikipedia.org), but with today's graphic arts capabilities, the value has surely increased. Science often uses the written word to generate mental pictures of a dynamic process. Dr. Henderson's artistic ability continues to provide us with excellent and aesthetically satisfying tools for the understanding of increasingly complex biological systems.

Author Index

Abstracts oral presentations

PAST SUCCESSES AND FUTURE CHALLENGES IN TARGETING KEY PLAYERS OF HIV PATHOGENESIS

Françoise Barré-Sinoussi

Unité de Régulation des Infections Rétrovirales, Institut Pasteur, Paris, France

FUNCTIONS OF THE TY3 RETROTRANSPOSON NC PROTEIN

Suzanne Sandmeyer

Department of Biological Chemistry, University of California, Irvine, Irvine CA 92697, USA

Ty3 is a Metavirus of the yeast, Saccharomyces cerevisiae. Two long terminal repeats (LTRs) flank an internal sequence containing GAG3 and POL3 open reading frames encoding Gag3 and Gag3-Pol3 proteins with domains homologous to capsid, spacer (SP), nucleocapsid (NC), protease, reverse transcriptase, and integrase. Ty3 expression is induced in mating yeast by exposure to pheromones. Ty3 RNA and proteins accumulate in intracellular foci (Beliakova-Bethell et al. 2006) or retrosomes. Viruslike particle (VLP) formation is associated with the appearance of retrosomes, which we propose are sites of VLP assembly.

Ty3 RNA and protein contain features are required for proper localization and assembly. Substitutions of conserved residues in the NC zinc finger motif resulted in loss of RNA foci and accumulation of Gag3 in the nucleus. Substitution of charged residues in SP resulted in failure of VLP condense and mature. In contrast, complete deletion of SP arrested retrotransposition subsequent to reverse transcription. We propose that the Ty3 NC zinc finger interacts with the RNA to mediate retrosome formation and with the NC basic domain interacts with the acidic domain of SP to condense VLPs (Clemens et al. 2011). Ty3 mutagenesis showed that Ty3 untranslated regions (UTRs) flanking GAG3 or heterologous UTRs flanking GAG3-POL3 were sufficient for concentration of RNA in retrosomes but GAG3 sequences were not. Together observations suggest that Ty3 UTRs as well as sequences within POL3 promote concentration of Ty3 RNA.

Mass spectrometry coupled with genomewide screens of the yeast deletion collection identified host proteins important for formation of retrosomes, assembly of Ty3 VLPs and retrotransposition. These proteins include members of RNA processing (P) bodies and stress granule complexes. We hypothesize that as yeast cells express Ty3 and arrest in G1 in response to mating pheromone, retrosomes containing components of P bodies and stress granules form, and that these promote multiple aspects of Ty3 VLP formation.

1. Beliakova-Bethell N, Beckham C, Giddings TH, Jr., Winey M, Parker R, Sandmeyer S. 2006. Virus-like particles of the Ty3 retrotransposon assemble in association with P-body components. RNA **12**(1): 94-101.

2. Clemens K, Larsen L, Zhang M, Kuznetsov Y, Bilanchone V, Randall A, Harned A, Dasilva R, Nagashima K, McPherson A et al. 2011. The Ty3 Gag3 Spacer Controls Intracellular Condensation and Uncoating. J Virol.

TRANSLATION AND LOCALIZATION OF HIV GENOMIC RNAS

Ricardo Soto-Rifo[1,2], Taran Limousin[1,2], Didier Décimo[1,2] and Théophile Ohlmann[1,2]

[1] INSERM U758, Lyon, F-69364 France; [2] Université de Lyon, Ecole Normale Supérieure de Lyon, Lyon, F-69364 France

The Human Immunodeficiency virus (HIV) genomic RNA (gRNA) plays a critical dual role in the cytoplasm of infected cells being used both as the packaged genome and as messenger RNA to produce Gag and Gag-Pol proteins. Over the last few years, a significant number of studies have reported how the genomic RNA could initiate translation and this lead to the conclusions that both cap-dependent and IRES-dependent translation were at use to synthesize viral proteins. However, only a few studies have focused on where translation of the genomic RNA could take place within the cell. Thus, to investigate this further, we have used cell imaging and functional assays to determine cytoplasmic localization of the HIV gRNAs. Our results show that the vast majority of the HIV-1 genomic RNAs were localized at the plasma membrane whereas the HIV-2 gRNAs accumulate in large cytoplasmic foci containing many stress granules components such as G3BP and TIAR. Interestingly, functional analysis showed that localization of the HIV-2 gRNA in cytoplasmic granules is determined by its association to polysomes and controlled by the levels of Gag polyprotein. Together, our results describe an unknown replication intermediate during the HIV-2 replication cycle which occurs after gRNA translation and before viral particle assembly.

THRIVING UNDER STRESS: SELECTIVE TRANSLATION OF HIV-1 STRUCTURAL PROTEIN mRNA DESPITE VIRUS IMPAIRMENT OF GLOBAL TRANSLATION

Sharma, A[1], Yilmaz, A[1], Marsh, K[2], Cochrane A[2], and K Boris-Lawrie[1]

[1]Department of Veterinary Biosciences; Center for Retrovirus Research; Center for RNA Biology; Comprehensive Cancer Center, Ohio State University, Columbus Ohio, USA; [2]Department of Molecular Genetics, University of Toronto, Toronto, Canada

All retroviruses utilize a single proviral transcription product to generate the viral genomic RNA and several mRNA templates for synthesis of all viral proteins. This functional versatility is made possible by host proteins that build functionally diverse retroviral ribonucleoprotein complexes (RNPs), which regulate the metabolism of the viral RNA. The characterization of the host components of these viral RNPs is necessary to understand this important virus-host interface and to design novel antiviral strategies. Herein RNA co-precipitations determined similar components of the RNP associated with spliced viral mRNAs and cellular mRNAs. However, distinct RNP components are observed to be associated with unspliced gag RNA, indicating a fundamental difference in this viral RNP. Result of metabolic labeling and polyribosome analysis determined this viral RNP is resistant to downregulation of cellular translation by pharmacological stress or by viral accessory protein. The investigation defined a novel strategy by which HIV-1 sustains translation of unspliced viral mRNA during impairment of global eIF4E-dependent translation. Our results uncover a previously unappreciated virus-host interface in mRNA translation. The outcome on the replication efficiency of HIV-1 will be discussed.

THE HIV-1-INDUCED BLOCKADE TO STRESS GRANULE ASSEMBLY: DECIPHERING THE ROLES FOR VIRAL AND NC-BINDING HOST FACTORS

Fernando Valiente-Echeverría*, Kishanda Vyboh*, Luca Melnychuk and Andrew J. Mouland

Lady Davis Institute for Medical Research and McGill UNiversity, Montreal, Québec, Canada

Following HIV-1 infection, the host responds by mounting a robust, anti-viral response in order to create an inhospitable environment for viral replication. As a countermeasure, in order to maintain viral protein synthesis and production, viruses may induce a block to the assembly of stress granules (SGs), that are translationally silent ribonucleoproteins (RNPs) and sites of RNA triage. We have previously shown that HIV-1 engineers the assembly of Staufen1-containing RNPs known as SHRNPs [J. Cell. Sci. 123:369 (2010)]. SHRNPs form in a HIV-1-dependent manner, harbour both Gag and the genomic RNA but do not contain canonical SG factors such as PABP and eIF3 as well as others. At the same time, HIV-1 expression imposes a blockade to the assembly of SGs in cells. In this work, we have mapped the viral determinants that mediate the HIV-1-induced blockade to SG assembly. Moreover, immunoprecipitation and proteomic analyses have allowed us to identify the host cell factors involved. The study on how HIV-1 counters the host's antiviral stress responses will contribute to our understanding of HIV-1 pathogenesis.

This work is supported by grants from the Canadian Institutes of Health Research and the Canadian Foundation for AIDS Research; F.V.-E. is supported by a Postdoctoral Fellowship from Becas Chile.

POST-TRANSCRIPTIONAL REGULATION OF GAG IN GAMMARETROVIRUSES

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Retroviruses compress large quantities of information into relatively small genomes. This is done by exploiting a number of mechanisms, including the utilization of unspliced mRNAs to produce essential viral proteins. Different classes of retrovirus have evolved unique mechanisms for exporting unspliced mRNAs out of the nucleus in such a way that they are efficiently translated. The lentivirus HIV-1, for example, encodes a trans-acting protein, Rev, that binds to a cis-acting RNA sequence in the unspliced mRNA called the Rev-response element; Rev then links the RNA to the CRM1-mediated export pathway. In analogous fashion, the cis-acting CTE in MPMV mRNA recruits the heterodimeric export factor NXF1/NXT1. How unspliced gammaretroviral mRNAs are exported and translated is not known. In the course of developing XMRV expression vectors, Gag polyprotein ($Pr65^{Gag}$) synthesis from a gag–only expression plasmid was found to be >20-fold lower than from a gag-pol expression plasmid. Mutations disrupting the pol open reading frame did not decrease $Pr65^{Gag}$ production, consistent with pol sequences acting in cis, at the level of the RNA. To map pol sequences required for activity, pol deletion mutations were generated. Two non-overlapping pol fragments of 300 and 700 nucleotides were each sufficient to stimulate $Pr65^{Gag}$ production. qRT-PCR assay of total, nuclear, and cytoplasmic RNA fractions revealed little change in the level of gag mRNA due to the presence of pol sequences. Nuclear retention or mRNA instability, then, do not explain poor $Pr65^{Gag}$ production in the absence of pol sequences. In ongoing experiments the importance of pol sequences for gag mRNA loading on to polysomes will be addressed, and attempts will be made to identify cellular proteins that associate with the pol fragments. These experiments provide what is perhaps the first detailed analysis of post-transcriptional regulation in gammaretroviruses.

GAG NUCLEAR TRAFFICKING AND VIRAL RNA PACKAGING

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The Gag protein of Rous sarcoma virus is unique among the orthoretroviruses for its intracellular trafficking pathway. After synthesis in the cytoplasm on free cytosolic ribosomes, RSV Gag enters the nucleus through an importin-mediated process. Once in the nucleus, the importins are displaced and Gag then binds to the CRM1/RanGTP complex for export from the nucleus. After nuclear egress, Gag is transported to the plasma membrane for release from cell via budding. A body of work from our laboratory has demonstrated a genetic link between the nuclear localization of Gag and genomic RNA incorporation, suggesting that Gag may select the genome for packaging within the nucleus.

We are now focused on elucidating the mechanistic details that underlie this complicated nucleocytoplasmic trafficking pathway and we wish to determine how the genome is recognized by the NC domain of Gag within the nucleus. We are taking a multidisciplinary approach, employing structural, genetic, and cell imaging methodology to address these fundamental questions. Using SAXS analysis, we determined that the protein is monomeric in solution and adopts a very compact structure, even more than HIV-1 Gag. The compact structure may be important to restrict access of targeting domains until the proper time and within the right location.

Upon binding to nucleic acids, RSV Gag undergoes a conformational change and forms dimers. Increasing the concentration of Gag leads to the stepwise formation of tetramers and hexamers that oligomerize in units of 6, with the hexamer as the basic unit. The structure of *in vitro* assembled Gag-nucleic acid hexameric subunits closely resembled the spacing and arrangement of hexamers examined in immature particles released from cells. These results suggest that the *in vitro* assembly of Gag-nucleic acid hexamers occurred with similar geometry as authentic hexamers in the fully-assembled lattice. Larger multimeric complexes were formed spontaneously by Gag when bound to ψRNA, with complexes up to 70 hexamers observed, although the most stable sizes were 3 and 7 hexameric units. Using nanogold to label ψRNA, we found that there was usually one nanogold particle in each complex of 1, 2, or 3 hexamers. Because each of the 18 individual Gag proteins in a 3-hexamer complex is unlikely able to make direct contact with the 100 nt ψRNA, we conclude that binding of Gag to the ψRNA sequence is a nucleating event that facilitates the cooperative addition of additional Gag proteins into a polyhexameric array through protein-protein interactions. Addition of the dimerization initiation site to ψRNA resulted in very large assemblages (~1400 Gag molecules), indicating that complexes the size of an immature particle were formed. Although the oligomerization of Gag occurs spontaneously *in vitro*, there appear to be forces that restrict the size of Gag:ψRNA complexes in the cell, particularly within the nucleus. We are testing the intriguing idea that CRM1:RanGTP binding restricts the size of the Gag:ψRNA complex in the nucleus to a dimer or tetramer, and polyhexamerization occurs after disassociation of the export factors and upon arrival at the plasma membrane.

DIFFERENCES IN ASSEMBLY PROPERTIES OF MLV AND HIV-1 GAGS

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We have characterized recombinant Gag proteins of HIV-1 (Datta, JMB 2007a, b) and MLV (Datta, submitted). We found several striking differences between them. One difference is that HIV-1 Gag dimerizes with a Kd of approximately 0.01 mM, while oligomerization of MLV Gag is barely detectable: it appears to dimerize with a Kd near 0.4 mM. In vivo, detergent-stripped immature MLV particles are disrupted by RNase (Muriaux, PNAS 2001) while those of HIV-1 are not (Campbell, PNAS 2001, Klein, JV 2011). Finally, HIV-1 Gag lacking NC can assemble into (somewhat aberrant) particles in vivo (Ott, JV 2003) while MLV Gag lacking NC does not assemble (Muriaux, JV 2004), even at extremely high expression levels (unpublished). We find that the HIV-1 dNC particles contain little, if any, RNA. All these results suggest that HIV-1 assembly is significantly less dependent upon RNA than MLV assembly. We asked whether this is because HIV-1 Gag has a far stronger dimer-interface than MLV Gag. Indeed, while HIV-1 Gag with a "WM" mutation ablating its dimer-interface, like dNC HIV-1 Gag, assembles fairly well in vivo (Datta JMB 2007), the double mutant WM/dNC is completely incapable of particle assembly. Similar observations were made with mutations in the zinc-finger cysteines, rather than total deletion of NC. We also see intracellular assembly of HIV-1 Gag lacking the myristylation site or with mutations in the highly basic region in MA, but not if these changes are paired either with mutations in the dimer interface or with dNC. Thus HIV-1 Gag will form (somewhat imperfect) particles as long as it has any 2 of these 3 functional domains: membrane-binding; dimer-interface; and RNA-binding. We conclude that these 3 domains are functionally redundant with respect to particle assembly.

PHOSPHOINOSITIDES IN RETROVIRAL ASSEMBLY

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Retroviral assembly occurs mainly at the plasma membrane upon the oligomerization of the viral Gag proteins. We and several research groups have participating in identifying specific membrane microdomains on which Gag assemble, such as raft domains or tetraspanin enriched microdomains. The targeting of Gag to the plasma membrane is favored by the myristate and the recognition of a specific phospholipid, the PI(4,5)P2, by basic residues in the MA domain of Gag. However some questions remain open: is Gag recruited in preexisting plasma membrane PI(4,5)P2 microdomains? Is Gag able to partitionate PI(4,5)P2 into Gag/PI(4,5)P2 microdomains? Is cellular PI(4,5)P2 localization and dynamics changing upon Gag expression in living cells? Are other motives in MA involved in Gag/membrane interactions?

We will try to answer some of these questions by multidisciplinary approaches including biochemistry, viral protein/membrane interactions, quantitative fluorescence microscopy, and cell biology.

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INTERACTION OF THE HIV GAG WITH VIRAL AND CELLULAR PARTNERS: *C*HARACTERIZATION OF THE COMPLEXES BY FLUORESCENT MICROSCOPY

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During formation of HIV particles, the Gag polyproteins are thought to interact with Vpr proteins to promote their encapsidation in the nascent particles [1]. To directly visualize and monitor the formation of the Gag-Vpr complexes and correlate their formation with Vpr oligomerization, we used two photon lifetime imaging microscopy (FLIM) [2] and time laps microscopy on HeLa cells expressing eGFP-labelled Vpr and tetracystein-tagged Gag proteins detected by the biarsenical ReAsH labelling reagent. Using these fluorescent microscopy approaches, we found that Gag proteins interact directly with Vpr proteins, which results in their transfer from the nuclear to the plasma membrane [3]. Vpr oligomerization was found critical for its interaction with Gag proteins while Gag mutants presenting polymerization failure were still able to interact with Vpr. Moreover, using PALM and TIRF microscopy we found a direct correlation between Vpr oligomerization and its localisation within budding particles. Taken together, these data show that Vpr encapsidation into nascent particles requires the recognition of Vpr oligomers by the C terminus of Gag.

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UNEXPECTED DEGRADATION OF NON VIRAL NUCLEIC ACIDS BY THE HIV-1 NUCLEOCAPSID PROTEIN: IMPLICATIONS FOR PACKAGING

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The HIV-1 nucleocapsid protein (NCP) is known to be involved in several obligate steps of the HIV replication cycle. Among other functions, it facilitates HIV-1 genome recognition, dimerization and encapsidation. The specific recognition of the HIV-1 Ψ packaging signal by the Gag polyprotein via its NC domain is well characterized. However, the mechanism of the enrichment of retroviral genomic RNA in the developing virions is still poorly understood. During viral packaging within an infected host cell, high localized concentrations of NCP are attained in the host cell cytosol. This, coupled with the fact that NCP engages in non-specific binding to nucleic acids, prompted us to investigate the effect of high (i.e. mM) concentrations of NCP on non-HIV-1 genome nucleic acids. We report here the ability of mM concentrations of HIV-1 NCP to degrade double stranded DNA. The degradation was abrogated when NCP devoid of zinc was used, or when the reaction was conducted in the presence of a NCP-nucleic acid binding inhibitor. HIV-1 and SIV Gag behaved similarly. In contrast to the effect that these proteins had on DNA, HIV-1 stem loop 3 (SL3) RNA was not degraded in their presence. However, HIV-1 NCP was observed to degrade mice salivary gland total RNA. Our findings hint at a possible means by which discrimination between host cell nucleic acids and the viral RNA genome is enhanced during viral packaging: namely the destruction of extraneous host-cell-derived nucleic acids during virus assembly.

STRUCTURE-FUNCTION RELATIONSHIP OF THE SEQUENCES NECESSARY FOR MOUSE MAMMARY TUMOR VIRUS (MMTV) gRNA DIMERIZATION AND PACKAGING

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MMTV is an endogenous retrovirus that causes breast cancer and leukemia in mice. MMTV is unique from other retroviruses in having multiple viral promoters which can be regulated by hormones in a tissue specific manner. This unique property has led to increasing interest in studying MMTV replication with the hope of developing MMTV based vectors for human gene therapy. However, not much is known about the packaging determinants of MMTV that allow the virus to specifically incorporate its gRNA into the virus particle over cellular and spliced RNAs. Employing an in vivo packaging and transduction assay, our deletion analysis of the 5'end of MMTV gRNA suggested that the entire 5'UTR in conjunction with the first 120 bp of Gag constitute the packaging determinants of MMTV gRNA. In an attempt to establish any structure-function correlation of the proposed MMTV gRNA packaging determinants, we folded the sequences from R up to the first 120 bp of Gag using RNA folding programs to predict the folding patterns of RNA molecules. Analysis of the predicted structures revealed that the 5'end of the MMTV gRNA folded into several stable stem-loops (SLs). In order to study the biological significance of this predicted structure, we performed mutational analyses of different structural motifs and tested the effects of these mutations on MMTV gRNA dimerization, packaging, and propagation using in vitro and in vivo approaches. Initial tests of these mutants revealed that perturbations in the predicted structural motifs greatly impinged crucial steps in MMTV life cycle including gRNA dimerization, packaging and propagation. The detailed results of the mutational analysis will be presented, which will further validate the secondary structure of the MMTV RNA packaging signal as well as delineate the role of the sequences needed for MMTV gRNA dimerization and packaging.

HOST CELL FACTORS IN RETROVIRUS ASSEMBLY AND RELEASE

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Expression of HIV-1 Gag is sufficient for the formation of virus-like particles. While it is well established that Tsg101 and other ESCRT components are required for HIV-1 budding/release, how Gag is transported to the site of assembly at the plasma membrane remains to be elucidated. We previously reported that the plasma membrane lipid $PI(4,5)P_2$ and the ADP ribosylation factors (Arfs) are crucial cellular factors for Gag localization to the plasma membrane. We also reported recently that disruption of SNARE proteins impairs Gag-membrane association. In ongoing studies, we identified novel cellular cofactors that are potentially involved in the late events of retroviral replication. Growth arrest-specific 7 (GAS7) and proline-serine-threonine phosphatase interacting protein 1 (PSTPIP1) are related proteins that harbor a conserved F-BAR domain that deforms membranes. We observed that GAS7 interacts directly with Tsg101 and the capsid (CA) region of HIV-1 Gag via its F-BAR domain and is incorporated into virions. Incorporated GAS7 is cleaved in virions by the viral protease. We determined that exogenous PSTPIP1 expression in PSTPIP1-deficient producer cells (1) severely impairs virion infectivity, (2) disrupts Env glycoprotein precursor (gp160) processing, (3) reduces cell-associated CA levels, and (4) impairs production of progeny virions. The reduction in cell-associated CA levels induced by PSTPIP1 overexpression likely reflects impaired Gag trafficking, in turn disrupting virus release. Importantly, endogenous PSTPIP1 and GAS7 are expressed exclusively in physiologically relevant target cells such as T-cells and monocyte-derived macrophages. We observe that endogenous PSTPIP1 in Jurkat T-cells accumulates at cell-to-cell contact sites and uropod structures, where HIV-1 Gag reportedly localizes during cell-to-cell virus spread, suggesting that PSTPIP1 may play a role in modulating cell-cell transmission. Together, these results suggest that F-BAR proteins may be important cellular cofactors in HIV-1 assembly and cell-cell virus propagation.

CELLULAR FACTORS THAT CONTRIBUTE TO HIV-1 POST-TRANSCRIPTIONAL GENE EXPRESSION

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Post-transcriptional regulation of HIV-1 gene expression is mediated by interactions between viral transcripts and viral / cellular proteins. For HIV-1, post-transcriptional nuclear control allows for the export of intron-containing RNAs which are normally retained in the nucleus. Specific signals on the viral RNAs, such as instability sequences (INS) and Rev responsive element (RRE), are binding sites for viral and cellular factors that serve to regulate RNA-export. The HIV-1 encoded viral Rev protein binds to the RRE found on unspliced and incompletely spliced viral RNAs. Binding by Rev directs the export of these RNAs from the nucleus to the cytoplasm. Previously, Rev co-factors have been found to include cellular factors such as CRM1, DDX3, PIMT and others. In this presentation, I will discuss new cellular factors that are discovered to influence HIV-1 transcriptional and post-transcriptional gene expression.

MOV10 ANTIRETROVIRAL MECHANISM

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MOV10 is a 1,003-amino acid putative RNA helicase that has seven helicase motifs in the C-terminal 521-911 region. It is a newly identified antiretroviral host factor, which is packaged into human immunodeficiency virus type 1 (HIV-1) virions via an interaction with the nucleocapsid (NC) domain of Gag, and inhibits viral replication from the next round of life cycle at a post-entry step. Here, we studied MOV10 packaging mechanism. We found that MOV10 packaging requires the NC basic linker, and HIV-1 Gag binds to the N-terminal 261-305 region of MOV10. Our predicted MOV10 three-dimensional model indicated that this region is located in a structurally exposed Cys-His-rich (CH) domain. Notably, although MOV10-Gag interaction is required, it is not sufficient for MOV10 packaging into the virions. This packaging also requires the C-terminal helicase motifs. In addition, we have mapped the minimum MOV10 anti-HIV-1 region to amino acids 99-949. In this region, we found that residues C188, C195, H199, H201, and H202 in the CH-domain as well as residues C947, P948, and F949 at the C-terminus critically regulate MOV10 anti-HIV-1 activity. Thus, we have identified two critical determinants for MOV10 packaging, and several other critical residues for MOV10 anti-HIV-1 activity. These results provide a molecular basis for understanding MOV10 antiretroviral activity.

EVIDENCE THAT GAG PROMOTES THE CYTOPLASMIC ANNEALING OF $tRNA^{LYS3}$ TO HIV-1 VIRAL RNA.

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In protease-negative HIV-1 (Pr(-)), the amount of $tRNA^{Lys3}$ annealed by Gag is modestly reduced (≈25%) compared to that annealed by mature nucleocapsid (NCp7) in protease-positive HIV-1 (Pr(+)). However, the $tRNA^{Lys3}$ annealed by Gag also has a strongly reduced ability to initiate reverse transcription, and binds less tightly to viral RNA. Transient exposure of Pr(-) viral RNA to NCp7 in vitro returns the quality of $tRNA^{Lys3}$ annealing to Pr(+) levels. The presence of APOBEC3G (A3G) both prevents this in vitro rescue, and creates a further reduction in $tRNA^{Lys3}$ annealing. Since A3G inhibition of NCp7-facilitated $tRNA^{Lys3}$ annealing in vitro requires the presence of A3G during the annealing process, these results suggest that in Pr(+) viruses, NCp7 can displace Gag-annealed $tRNA^{Lys3}$, and re-anneal it to viral RNA, the re-annealing step being subject to A3G inhibition. Together, these data support the possibility that the initial annealing of $tRNA^{Lys3}$ in Pr(+) virus is facilitated by Gag at the pre-budding stage, promoted by the unique architecture of an early, pre-processed HIV-1 assembly intermediate, with fine-tuning of annealing occurring after precursor proteolysis. Direct evidence for the cytoplasmic annealing of $tRNA^{Lys3}$ to viral RNA will be presented by demonstrating the presence of specific $tRNA^{Lys3}$ fragments in the cytoplasm of HIV-1 infected cells, the appearance of which depends upon the ability of $tRNA^{Lys3}$ to be annealed to the viral RNA.

IDENTIFICATION AND CHARACTERIZATION OF GAG-BINDING CELLULAR COFACTORS REQUIRED FOR HIV-1 REPLICATION

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Like other viruses, HIV-1 replicates by usurping numerous cellular pathways, including RNA polymerase II transcription, RNA export, and the ESCRT machineries involved in vesicular trafficking. These interactions extend the capabilities of the virus and are critical for viral replication. To identify additional cellular cofactors that interact with the HIV-1 structural proteins, we used the viral Gag, MA, CA and NC proteins as "baits" in affinity purification/mass spectrometry analyses. Candidate cellular cofactors were ranked using a novel quantitative scoring system, and the top 24 proteins were tested for functional importance in viral replication using an siRNA depletion approach and for binding to recombinant Gag-derived proteins *in vitro*. Three of the candidate proteins reduced viral replication greater than 20-fold when depleted from cells. All three of these candidates bound the viral NC protein, with two binding in an RNA-dependent fashion. Additional studies indicate that all three factors act during late stages of the viral life cycle, and each appears to affect a different step in viral replication, including RNA splicing, virus assembly, and maturation. We are currently characterizing the cofactors mechanisms of action further, and my presentation will describe the factors and our current understanding of their roles in HIV-1 replication.

THE ROLE OF NC IN VIRUS BUDDING

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Retroviruses carry a Nucleocapsid (NC) domain in their structural protein Gag, which has been endowed with numerous functions including RNA encapsidation, reverse transcription and virus assembly. We recently extended this list with one novel function that involves NC in virus budding. We found that mutations in NC eliminated virus release of HIV-1 and EIAV. In both cases, NC mutant viruses displayed clear budding defects as fully assembled particles remained tethered to the cell surface with long membranous stalks. These findings suggested that a functional NC is required for viruses utilizing divergent Late (L) domain motifs. Careful examination of the role of NC in Alix-mediated virus budding uncovered an interaction between NC and Alix N-terminal Bro1 domain. Ectopic expression of Bro1 rescued the release of an HIV-1 virus lacking all known L domain motifs. This rescue required the ESCRT-III CHMP4 binding site in Bro1, suggesting that NC-Bro1 interaction mediates the recruitment of the down-stream acting ESCRT-III members required for virus fission from the cell. A structure-function analysis of Bro1 role in HIV-1 release revealed the requirement for an extended loop centered at residue Phe105. Mutation of residues at the tip of the Phe105 loop obliterated Alix function in HIV-1 release without interfering with its ability to bind Gag or engage its natural partner CHMP4. These studies describe a new role for NC in virus release and reveal that NC is involved in the recruitment of cellular factors that facilitate membrane fission events critical for virus release.

SERENDIPITY BEHIND THE SEARCH FOR RETROVIRAL NUCLEOCAPSID STRUCTURES AND FUNCTIONS

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Retroelements, namely retrotransposons and retroviruses, are widespread in all eukaryotes from yeast to man where they are found as abundant remnant sequences as well as circulating particles seldom infectious. Retroelements replicate by a process of RNA reverse transcription – DNA integration, in a similar manner from yeast transposon to HIV-1. The molecular machine governing this dual replication process is the nucleocapsid structure, resembling a ribonucleoparticle where the RNA and nucleocapsid protein molecules are the major interacting components.

The nucleocapsid (NC) of HIV-1, and that of gamma and alpha-retroviruses, has been the subject of intense interest and research, and due to that a large number of studies on NC structure and functions from macromolecular structures to virology and drug discovery has been published (more than 1500 articles for retroviruses and 70 for retrotransposons).

I will briefly describe how serendipity or chance influenced our research on NC during the past thirty years or so, notably the original observation that NC protein of ASLV was interacting with the genomic RNA (but not the Matrix protein) ; the discovery that NC protein was chaperoning viral DNA synthesis, from initiation to completion, protection and maintenance, and ultimately its integration ; a novel understanding on how a protein such as NC protein can achieve multiple functions in retrovirus replication, probably as a IUP (intrinsically unstructured protein) endowed with oustanding talents in macromolecular interactions and their dynamics ; the realization that NC-like proteins are widespread in the virus world, eg in Flaviviruses, Orthomyxoviruses, Hepadnaviruses, and to a certain degree in living cells, possibly acting as an anti-viral restriction factor. But it remains that the assembly, composition, structure and dynamics of such a macromolecular ensemble are yet poorly understood. And drugs specifically targeting HIV-1 NC protein are still to be discovered.

Review. J.-L. Darlix, J. Godet, R. Ivanyi-Nag, P. Fossé, O. Mauffret and Y. Mély Flexible Nature and Specific Functions of the HIV-1 Nucleocapsid Protein. J. Mol. Biol. (2011) 410, 565–581

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HIV-1 GAG BINDS WITH HIGH AFFINITY AND SPECIFICITY TO PSI RNA IN VITRO

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How retroviral Gag proteins specifically select their RNA genomes for packaging in the presence of a vast excess of cellular RNA is not well understood. In this work, we quantify HIV-1 Gag and nucleocapsid protein (NC) binding to two ~100-nt RNAs derived from the HIV-1 genome—Psi (nt 234-329) and TAR/polyA (nt 1-104). We performed competition-binding studies in which fluorescently-labeled RNA pre-bound by Gag or NC is displaced by increasing concentrations of Na^+ cations. These experiments allow us to characterize the salt-dependence of the dissociation constant, $K_{d,[Na+]}$, and to calculate the net effective positive charge of the protein (Z_{eff}), the non-electrostatic component of the protein/RNA interaction ($K_{d,1M}$), and the dissociation constant at physiological salt concentrations ($K_{d,150mM}$). Our studies suggest that the effective positive charge of NC (Z_{eff}=3.7±0.5) is similar for binding to both RNAs tested. In addition, at 150 mM NaCl, NC binds Psi with ~50-fold higher affinity than TAR/polyA (42 nM vs. 2 mM, respectively). The Z_{eff} for Gag binding to Psi and TAR/poly A is 5.2±0.4 and 10±0.2, respectively, which may reflect different contributions of the MA domain. At 150 mM NaCl, Gag has an ~140-fold higher binding affinity for Psi relative to TAR/polyA (K_d=780 pM and 110 nM, respectively). Importantly, for both NC and Gag, the non-electrostatic contribution to binding Psi is much greater than for binding to TAR/polyA. These data strongly support a specific binding interaction with Psi, in contrast to primarily electrostatic binding to TAR/polyA. Gag binds with significantly reduced affinity to a truncated Psi RNA variant that lacks both SL2 and SL3. A single-point mutation to either zinc finger greatly reduces the binding affinity of Gag for Psi. Taken together, the in vitro studies reported here provide new insights into how HIV-1 Gag achieves its exquisite vRNA binding selectivity in vivo.

ROLE OF NC IN REVERSE TRANSCRIPTION-DRIVEN UNCOATING OF MATURE HIV CAPSID

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Until recently, it was a common notion that reverse transcription (RTion) in retroviruses, including HIV, happens within the cytoplasm of the infected cell after mature capsid uncoating. However, recent studies have shown that the optimum stability of the mature HIV capsid is critical for viral infectivity {Aiken}. Moreover, it was shown in vivo that the capsid remains intact during most of RTion, and that RTion stalling significantly slows down uncoating {Hope}. In this theoretical work, we consider the problem of mature HIV capsid uncoating driven by polymerization of double-stranded (ds) viral DNA by reverse transcriptase (RT). We take into account the millimolar concentration of NCp7 contained within the capsid, which aggregates both single-stranded (ss) RNA and dsDNA when the capsid is intact. NC-aggregated, flexible ssRNA occupies only a small fraction of capsid volume. In contrast, NC-aggregated, rigid dsDNA is expected to form a tightly wound toroid that will become comparable to or larger than the capsid cross-section upon reaching 10^3-10^4 base pairs (bp) in length. At this point, either the toroid or the capsid shell will deform, depending on their comparative rigidities. Since the experimental elasticity parameters of the mature HIV capsid are not yet known, we consider both scenarios. The capsid is expected to break open (uncoat) when its stress reaches a critical value, as described by elasticity theory. Alternatively, the stress provided by the capsid on the growing toroid may stall RT. Importantly, we predict that a significant fraction of viral dsDNA can be polymerized by RT in the presence of NCp7 before uncoating, even for a low stability capsid. We suggest that lowering the pressure of the growing dsDNA on the capsid shell to prevent pre-mature uncoating is another critical function of NCp7 in the HIV lifecycle.

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SITE-SELECTIVE PROBING OF cTAR STRUCTURAL CHANGES INDUCED BY THE HIV-1 NUCLEOCAPSID PROTEIN

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The HIV-1 nucleocapsid protein (NCp7) is a nucleic acid chaperone facilitating reverse transcription. During the first strand transfer, the (-)DNA copy of TAR (cTAR) is destabilized by NCp7 which subsequently directs the TAR/cTAR annealing through a zipping pathway. Here, we selectively modified the 57-nucleotides cTAR of the Lai strain with 2-aminopurine (2-Ap) in different positions within its stem-loop structure to locally probe the structure and dynamics of cTAR in the absence and in the presence of NCp7. Time-resolved fluorescence studies revealed the structural and dynamical heterogeneity of cTAR. Addition of NC(11-55), a truncated version of NCp7 limited to the zinc-finger domain, was found to locally destabilize the stacking of the bases and to decrease the mobility of the cTAR segment where NC(11-55) is bound. We evidenced two preferential destabilized sites near the CA mismatch in the lower half of the cTAR stem. Our results provide insights towards a molecular description of the NCp7 destabilizing mechanism. They also highlight the necessary flexibility of NCp7 to adjust the sequence and structure variability to chaperone the TAR/cTAR annealing reaction through a particular reaction pathway.

CORONAVIRUS TRANSCRIPTION IMPLIES DISCONTINUOUS RNA SYNTHESIS THAT REQUIRES THE NUCLEOCAPSID PROTEIN

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Coronavirus (CoV) transcription implies a discontinuous RNA synthesis step during the of the production of the mRNAs by which a copy of the 5'-terminal leader sequence is fused to a minus RNA copy of the 5' end of the mRNA coding sequence (body) (1). Transcription-regulating sequences (TRSs) preceding each gene are also found at the 3'-end of the leader. Base pairing between the leader TRS (TRS-L) and the complement of the body TRS (cTRS-B) in the nascent RNA is a main determinant factor during CoV transcription. In transmissible gastroenteritis CoV (TGEV), a good correlation has been observed between subgenomic mRNA levels (sgmRNA) and the free energy of TRS-L and cTRS-B duplex formation, with the only exception of the sgmRNA of nucleocapsid (N) protein, the most abundant viral protein during CoV infection, in spite of the minimum free energy of the duplex formation. We have shown that sgmRNA N expression was specifically controlled by a transcription enhancer preceding the coding sequences of N gene (2), and that both TGEV and severe and acute respiratory syndrome CoV (SARS-CoV) N proteins have RNA chaperone activity (3). The location of the domain containing RNA chaperone activity was identified, and its mechanism of action during RNA synthesis analyzed. N protein and its deletion mutants with RNA chaperone activity enhanced template switch in a retrovirus-derived strand transfer system. We have proposed that the requirement of N protein for CoV transcription may be associated to its RNA chaperone activity, which could facilitate the transcriptase template switch required during the discontinuous RNA synthesis, to generate a negative copy of the viral sgmRNAs (4).

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THE CORONAVIRUS NUCLEOCAPSID PROTEIN IS AN RNA CHAPERONE

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Purified nucleocapsid (N) protein from transmissible gastroenteritis virus (TGEV) enhanced hammerhead ribozyme self-cleavage and favored nucleic acid annealing, properties that define RNA chaperones, as previously reported (3). The location of the domain containing RNA chaperone activity and its role during RNA synthesis was then analyzed. Several TGEV N protein deletion mutants were expressed in *Escherichia coli* and purified, and their RNA binding ability and RNA chaperone activity were evaluated. The smallest N protein domain analyzed with RNA chaperone activity, facilitating DNA and RNA annealing, contained the central unstructured region (amino acids 117 to 268). Interestingly, N protein and its deletion mutants with RNA chaperone activity enhanced template switching in a retrovirus-derived heterologous system, reinforcing the concept that TGEV N protein is an RNA chaperone that could be involved in template switching. The role of N protein during coronavirus RNA synthesis was analyzed *in vivo*, using a TGEV replicon system previously generated by our group (1). TGEV replicons containing full- length N protein or lacking N protein were used, and their activity in transcription and replication was analyzed by quantitative RT-PCR. N protein was not essential for replication, but it was required for an efficient transcription (2). These results were compatible with a potential role of N protein RNA chaperone activity in coronavirus transcription.

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NUCLEIC ACID CHAPERONE PROPERTIES OF THE HIV-1 TAT PROTEIN: COMPARISON WITH NCP7

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Though the main function of the HIV-1 Tat is to promote the transcription of the proviral DNA, Tat is also thought to be involved in reverse transcription (RTion) by a still unknown mechanism. The nucleic acid annealing activity of Tat might explain, at least in part, its role in RTion. To further explore this possibility, we carried out a fluorescence study on the mechanism by which the full length Tat(1-86) and the basic Tat(44-61) direct the annealing of the HIV-1 dTAR DNA and cTAR DNA sequences, essential for the early steps of RTion. Though both peptides were unable to destabilize the cTAR stem, they strongly promoted cTAR/dTAR annealing (1). This Tat promoted-annealing is nucleated through the thermally frayed 3'/5' termini, resulting in an intermediate with 12 intermolecular base pairs, which then converts into the final duplex. Moreover, Tat(1-86) appeared as efficient as NCp7, in promoting cTAR/dTAR annealing, and could act cooperatively with NCp7 during RTion. Using Tat(44-61) mutants, we evidenced that the Tyr47 residue and the basic amino acids in the central (50-51) domain play a key role in the nucleic acid binding and chaperone mechanism of Tat. Moreover, Tat(44-61) was also shown to promote the annealing of the (+)/(-)PBS sequences involved in the second strand transfer of RTion. However, in sharp contrast to NC, the Tat peptide was unable to induce the mechanistic switch in the (+)/(-)PBS annealing pathway that is likely required to ensure the specificity and fidelity of the second strand transfer. This mechanistic switch was related on the modifications of the PBS loop structure and dynamics that are strictly dependent on the integrity of the NC zinc fingers (2,3). Taken together, our data are consistent with a role of Tat in the stimulation of the obligatory strand transfers during RTion.

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THE HIV-1 VIF PROTEIN: OLIGOMERIZATION, RNA CHAPERONE ACTIVITY AND MORE …

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The HIV-1 viral infectivity factor (Vif) is a small basic protein essential for viral fitness and pathogenicity. Vif is required for productive infection of non-permissive cells, where it counteracts the antiviral activities of the cellular cytosine deaminases APOBEC3G (A3G) and A3F. Several mechanisms have been described to explain the reduction of AG in cells and in viruses: (1) degradation by the ubiquitin-proteasome pathway, (2) translational repression by Vif and (3) competition with other RNA/protein binding partners. Moreover, Vif is associated with the viral assembly complexes and seems to be packaged into viral particles through interactions with the viral genomic RNA and/or the nucleocapsid domain of Gag.

To address the role of Vif in viral assembly, we first characterized the impact of alanine substitutions in the putative multimerization domain of Vif (161PPLP/AALA164) on Vif oligomerization, secondary structure content and nucleic acids binding properties. We showed that wt Vif forms oligomers of 5-9 proteins, while Vif AALA forms dimers and/or trimers. Up to 40 % of the unbound wt Vif sequence appear to be unfolded, but binding to the HIV-1 TAR apical loop promotes formation of ß-sheets. Interestingly, Vif substitutions do not significantly affect its secondary structure, but they diminish its binding affinity and specificity for nucleic acids. In a second step, we directly studied the oligomerization of Vif in living cells by using a FRET approach analyzed by FLIM. As expected, we confirmed that Vif-Vif interactions occur in cells. Interestingly, when Vif is co-expressed together with Gag, Vif is largely re-localized to the cell membrane where Vif oligomerization still occurs. Finally, we tried to identify domains of Vif involved in its RNA chaperoning properties by analyzing the enhancement of in vitro HIV-1 RNA dimerization. We found that the C-terminal region of Vif possesses this activity. Interestingly, this region has been shown to be disordered suggesting it could easily interact with a range of many different partners. Oligomerization and chaperoning activities of Vif could be important for Vif functions involving RNAs.

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DIFFERENCES IN THE CONTRIBUTIONS OF THE ZINC FINGERS OF HIV-1 NC AND THE NC DOMAIN IN GAG DURING EARLY STEPS IN REVERSE TRANSCRIPTION

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Synthesis of (-) strong-stop DNA follows initiation of HIV-1 reverse transcription by $tRNA^{Lys3}$. While polymerization proceeds, RNase H degrades the viral RNA template and generates 5'-terminal RNAs (14-18 nt), which are annealed to the 3' end of the DNA. These RNAs must be removed to allow minus-strand transfer to occur. Terminal fragment removal and a role for NC duplex destabilization activity and/or RNase H have not been studied in detail. To address this issue, we modeled fragment removal in the context of minus-strand transfer. A 20-nt 5'-terminal RNA was heat-annealed to a synthetic (-) strong-stop DNA, followed by addition of HIV-1 NC, acceptor RNA, and RT; the amount of transfer product was then determined. The 20-mer had no effect on strand transfer, implying that it had been successfully removed. In the presence of the 20-mer, strand transfer efficiency was highest with WT RT and NC; with RNase H-minus RT and NC, the rate of transfer was reduced by two-fold. Mutational analysis also showed that removal is strongly dependent upon NC's ability to coordinate zinc. Direct evidence for fragment removal and the requirement for zinc coordination was obtained in EMSA experiments with labeled RNA. These data demonstrate the critical importance of NC's helix destabilization activity in the reaction immediately preceding strand transfer and are in excellent agreement with our findings on the fidelity of plus-strand DNA initiation[1]. We have also continued our investigation of the chaperone activity of Gag. Recently we observed that Gag promotes minus-strand transfer in vitro, indicating that it can weakly destabilize nucleic acid structures. However, high Gag concentrations severely inhibit DNA elongation ("roadblock" mechanism)[2]. To explore possible effects of zinc finger (ZF) mutations in Gag, we tested a set of mutants[3,4] in assays for minus-strand transfer and extension of annealed $tRNA^{Lys3}$. Surprisingly, the ZF mutants generally behaved like WT Gag. Collectively, our results demonstrate that NC's ZF motifs are critical for reactions requiring duplex destabilizing activity (e.g., minus-strand transfer), whereas in some of these same reactions, Gag's ZFs appear to have a less significant role.

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FROM MILLIONS TO ONE, PROBING THE EARLY STEPS OF HIV INFECTION

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HIV undergoes a high rate of mutation and recombination during reverse transcription, but it is not known whether these events occur independently or are linked mechanistically. Here we use a system of silent marker mutations in HIV and a single round of infection in primary T-lymphocytes, combined with a high-throughput sequencing and mathematical modelling approach to directly estimate the viral recombination and mutation rates. From >7 million nt of sequences from HIV infection, we observe 4801 recombination events and 859 substitution mutations (≈1.51 and 0.12 events per 1000 nt respectively). We use experimental controls to account for PCR-induced recombination and sequencing error, and find the single cycle virus-induced mutation rate is 4.6×10^{-5} mutations per nt after correction. By sorting our data into recombined and non-recombined sequences, we find a significantly higher mutation rate in recombined regions (p=0.003, Fisher's exact). We use a permutation approach to eliminate a number of potential confounding factors and confirm that mutation occurs around the site of recombination, and is not simply co-located in the genome. By comparing mutation rates in recombined and non-recombined regions we find that recombination-associated mutations account for 15-20% of all mutations occurring during reverse transcription. In an attempt to identify the functional reverse transcription complex to define the mechanism, we have developed a generic and highly sensitive (1-2 copies) approach (oligo-fluorescent *in situ* hybridization, O-FISH) to detect nucleic acid target sequences that are 24-26 nucleotides in length. Using synthesis of viral genome as a marker, O-FISH can be used as a general tool to discern infectious from non-infectious viruses. Furthermore, our O-FISH technology highlights the potential and means to visualize single copies of miRNA in cells.

TOWARDS A MECHANISTIC INSIGHT OF HIV-1 NC MATURATION

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Maturation of a HIV-1 virion by its protease results in establishing a condensed ribonucleoproteic architecture, i. e. the nucleocapsid, that internalize the retroviral RNA associated to the mature nucleocapsid protein (NCp7) within its surrounding capsid (1). Up to now, it is still poorly documented how this critical process is controlled, as well as how protease acts within HIV-1 virions to produce NCp7 from its Gag substrate

Here, by coupling TEM visualization of HIV-1 particles, AFM visualization of NC-ssDNA complexes and enzymology of PR-directed NC processing, we show that 1) NCp7 production by protease-driven Gag processing provokes RNA condensation following the secondary SP2-p6 cleavage step; 2) this step, as well as the primary cleavage step, SP1-NC, and the tertiary cleavage step, NC-SP2, are strongly dependent of the NC-RNA interaction and NC clustering along the viral RNA, leading to a Tarzan-like processive mechanism of proteolysis .

These reactions are optimal in vitro between pH 6 and 6,5, where efficient RNA or DNA binding to NC provokes a severe activation of PR to remove SP1, p6 and SP2. The principal contribution obviously consist in a dual effect of nucleic acid binding to its NC target: first, an allosteric switch of the NC surrounding structure leading to an easier access of PR to the flanking sites of the NC domain, followed by a macromolecular crowding effect provoking PR processivity due to neighbouring of NC domains along RNA or ssDNA strands.

These data drive us to a new vision of HIV-1 maturation with the impact as an up-regulator of Gag processing of the intravirion RNA leading to its own condensation by directing NC processing. The many consequences of this mode of action will be highlighted after presentation of our data, noticeably about the unexpected implication of RNA or DNA as a scaffold for a processive mechanism of proteolysis of nucleic-acid binding proteins.

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A PROPOSAL OF NEW STRUCTURAL MODEL OF HIV-1 DLS

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Very little is known about the overall structure of HIV genome dimerization site. Based on electron microscopy, the 5' region of both viral RNAs contains a primary contact point and is referred to as the dimer linkage structures (DLS). The presumptive primary DLS of HIV-1 has been mapped to a region near the major splice donor and biochemical analysis indicate that the DLS probably consists of multiple stem-loop structure. Within DLS, stem-loop 1 (SL1) has been regarded as the most important region, which forms a stem-loop structure with a hairpin loop containing a six-nucleotide palindromic sequence. The dimer formation would occur through a kissing hairpin mechanism by which the two RNAs would form an initial loop-loop contact based on complementary anti-parallel base pairing at the loops of SL1. We previously developed a system to assess the DLS operating within the HIV-1 virion and identified the region which is necessary and sufficient for HIV-1 genome dimerization in the virion (1, 2). By applying our system for fine mapping of DLS, we discovered the possibility of long-range interaction within DLS structure which has been never noticed previously. The only one-base substitutions to disrupt the interaction grossly reduced viral genome dimerization and viral replication. Furthermore, the compensation mutations within DLS to restore the interaction recovered the genome dimerization efficiency. We further validated the several stem formations of DLS including SL1 those have been suggested previously, and reconfirmed specific base pairings in some of them. We performed computer-assisted structure modeling with accumulated information, and obtained very unique two thermodynamically stable forms of the dimer of a minimum DLS element. The models are consistent with the above and previous experimental data and further suggest a structural basis for dimerization, stabilization, and interactions of the DLS element.

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PREMATURE REVERSE TRANSCRIPTION IS NOT THE CAUSE OF THE REPLICATION DEFECT IN NC-MUTANT HIV-1

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HIV-1's NC protein is critical for viral replication. It is involved in assembly, genome packaging, budding, maturation, reverse transcription, and integration. Two NC mutations, His23Cys and His44Cys cause premature reverse transcription resulting in virions with ~1,000-fold more vDNA than wild-type virus, and these mutants are replication defective with a key defect in integration. We successfully blocked premature reverse transcription by transfecting proviral plasmids into cells cultured in the presence of high levels of reverse transcriptase inhibitors. Examination of infectivity after removal of the inhibitors did not show a rescue of the infectivity defects in the NC mutants. Surprisingly, kinetic endogenous reverse transcription assays of the NC mutants functioned identically to wild-type when premature reverse transcription was blocked. However, infection of permissive cells showed that while prevention of premature reverse transcription in the NC mutants resulted in lower quantities of initial reverse transcripts, the reverse transcription kinetics were not restored to wild-type levels. Thus, the replication defects in the NC mutants are not the result of premature reverse transcription but an independent side-effect of the mutations.[1]

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DISSECTING THE ROLE OF HIV GAG'S ZINC FINGERS IN NUCLEIC ACID BINDING, CHAPERONE ACTIVITY AND PREMATURE REVERSE TRANSCRIPTION

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The assembly of HIV-1 proteins and viral RNA (vRNA) relies heavily on the multifunctional Gag polyprotein. Gag's zinc-finger (ZF) domains located in the NC domain are believed to specifically interact with the Psi vRNA packaging signal. Gag has also been shown to display NC-domain dependent chaperone activity. Recent findings have shown that mutation of the ZF's of Gag produces noninfectious virions containing largely DNA instead of RNA. Here we have analyzed the same Gag variants studied in the cell-based assays (H400C, H421C, H400/421C, DZF1, DZF2, and DZF1+2) to determine how these mutations alter Gag's interactions with nucleic acids (NA) and chaperone function in vitro.[1] We demonstrate that Gag's ability to aggregate and bind nonspecific NA is unimpaired by these mutations but that interaction with specific NA (i.e., Psi RNA) is reduced by at least 20-fold. Gag ZF variants are capable of catalyzing $tRNA^{Lys3}$ primer annealing and the TAR RNA/DNA annealing step of minus-strand transfer as effectively as WT Gag. The latter result is in contrast to mature NC ZF variants, which display reduced TAR annealing due to weaker strand destabilization activity. Our data are consistent with a primary role of Gag's ZF domains in Psi recognition, and do not support a critical role of the ZF structures in nonspecific RNA binding and chaperone activity. Based on our in vitro assays, we hypothesize that Gag ZF variants interact less effectively with the vRNA Psi sequence, leading to a reduction in the rate of assembly, which may result in premature reverse transcription.

1. For chaperone activities of the Gag mutants in minus-strand transfer, see also abstract by T. Wu et al.

PROTEOLYTIC MATURATION OF THE HIV-1 CORE

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STRUCTURAL INSIGHTS INTO THE MECHANISM OF HIV-1 GENOME SELECTION AND PACKAGING

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The 5'-untranslated region (5'-UTR) of the HIV-1 genome contains a conserved element spanning the 5'-UTR-gag junction (G328-A356, AUG) that is critical for genome dimerization and packaging and has been proposed to function as a regulatory element. We show by NMR that AUG adopts a hairpin structure in the monomeric 5'-UTR (356 nt; 115 kDa) and forms intramolecular base pairs with an upstream element (U5) in the dimer. U5:AUG formation promotes dimerization and enhances binding by the nucleocapsid protein (NC), the cognate domain of the viral Gag protein required for packaging. Mutations that stabilize the hairpin inhibit dimerization and NC binding in vitro and strongly attenuate RNA packaging in vivo, whereas mutations that enhance U5:AUG formation promote 5'-UTR dimerization, NC binding and RNA packaging. Our findings suggest that diploid genome selection is mediated by an RNA switch mechanism, in which conformational changes induced by U5:AUG formation expose residues that promote dimerization and NC binding. To gain insights into the structural basis for RNA recognition, we have assigned the NMR spectrum of a 156 "core packaging signal" from the 5'-UTR using a ^{2}H-edited NMR approach. This approach enabled unambiguous identification of sequential, long-range and cross-helix NOEs and facilitated the structure determination of the RNA.

NUCLEOCAPSID-ASSISTED FORMATION OF HIV-1 REVERSE TRANSCRIPTION INITIATION COMPLEX

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HIV-1 reverse transcriptase uses the host $tRNA^{Lys}_3$ as a primer for the synthesis of the minus DNA strand. The first event in viral replication is thus the annealing of the tRNA to the primer binding site (PBS) in the 5' UTR of the viral RNA. This event requires a major RNA rearrangement which is chaperonned by the viral NC protein. The binding of NC to nucleic acids is essentially non-specific, however, NC is known to bind selectively to hairpins located in the 5' region of the viral RNA. In a previous study, using an NMR approach in which the reaction is slowed down by controlling temperature, we were able to follow details in this RNA unfolding/refolding process and to uncover an intermediate state. We showed that the annealing can initiated both from the single-stranded CCA-3' end of the acceptor stem and from the bottom of the acceptor/TYC stem. Secondly, the complete annealing is reached only in the presence of NC, at physiological temperature, when the zinc fingers of NC contact the D loop of $tRNA^{Lys}_3$.
More recently, we have investigated by NMR the formation of the PAS/antiPAS interaction upon annealing of $tRNA^{Lys}_3$ to the PBS. Indeed, the PAS ('Primer activation signal) motif is not involved in tRNA placement, but enhances its usage as primer. The PAS-antiPAS interaction is controversial and our study shows that this interaction required the action of the nucleocapsid protein. Recent results will be presented.

STRUCTURAL INSIGHTS INTO THE cTAR DNA RECOGNITION BY THE HIV-1 NUCLEOCAPSID PROTEIN

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An essential step of the reverse transcription of the HIV-1 genome is the first strand transfer that requires the annealing of the TAR RNA hairpin to the cTAR DNA hairpin. HIV-1 nucleocapsid protein (NC) plays a crucial role in this process by facilitating annealing of the complementary hairpins. In our work we studied properties of NC(11-55) binding to the top half of the cTAR DNA (mini-cTAR) using nuclear magnetic resonance and gel retardation assays. Despite the large number of unpaired guanines in the sequence, we could show that NC(11-55) binds nearly exclusively at a unique site : the TGG sequence in the lower stem, this latter being destabilized by the adjacent internal loop. The 5' thymine in this sequence interacts with residues of the N-terminal zinc knuckle and notably Phe16 and the 3' guanine is inserted in the hydrophobic plateau of the C-terminal zinc knuckle and undertakes stacking interaction with Trp37. The TGG sequence is preferred relative to the apical and internal loops that contains unpaired guanines. Indeed, the former does not bind at all NC(11-55) under a various set of conditions. Analysis of the complex and comparison with the others resolved NC-DNA/RNA complexes permit to establish some interesting conclusions. The binding polarity, defined by the relative orientation of the 5' to 3' nucleic acid chain and of the N-to C terminal chain of the protein, is parallel in the DNA-NC complexes and antiparallel in the RNA-NC complexes. Detailed investigation of the DNA–protein contacts shows the major role of hydrophobic interactions involving nucleobases and deoxyribose sugars. A similar network of hydrophobic contacts involving deoxyriboses is observed in the NC:DNA complexes, whereas NC contacts ribose differently in NC:RNA complexes. We propose that the binding polarity of NC to nucleic acids could be closely related to these contacts.

STRUCTURAL DYNAMICS OF RETROVIRAL GENOMES

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Retroviral RNA genomes form myriad structures that are governed by critical interactions with either the nucleocapsid (NC) protein or the nucleocapsid domain of Gag polyprotein. We have created two powerful technologies, based on SHAPE (selective 2'-hydroxyl acylation analyzed by primer extension), to explore interactions between NC and the RNA genomes of the xenotropic murine leukemia virus related virus (XMRV) and the Moloney murine leukemia virus (MuLV). In the first approach, ultra-sensitive SHAPE, we developed a two-color capillary electrophoresis instrument with low attomole (10^{-18}) sensitivity. New analysis approaches and the high sensitivity of this instrument allowed us to obtain unprecedented single-nucleotide resolution structures from authentic RNA genomes of MuLV and XMRV. In the second approach, time-resolved SHAPE, we were able to create an RNA "movie" revealing the structural biogenesis of the MuLV retroviral genome as it binds to NC. The data generated using these SHAPE-based technologies allow us to propose detailed mechanisms for both the specific RNA binding and the chaperone activities of NC.

SHAPE ANALYSIS OF THE FIV PACKAGING SIGNAL RNA REVEALS A STRUCTURAL SWITCH POTENTIALLY CONTROLLING PACKAGING AND GENOME DIMERISATION

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Feline immunodeficiency virus (FIV) infects many species of cat, and is related to HIV, causing a similar pathology. The viral RNA contains an unusual packaging signal that spans two independent regions within the first 511 nucleotides of the genome. Previous studies of the packaging signal RNA showed four conserved stem-loops, extensive long-range interactions (LRI), and a small, palindromic stem-loop (SL5) within the gag open reading frame (ORF) that may act as a dimerisation initiation site (DIS), enabling the virus to package two copies of its genome. Here, we have used high-throughput SHAPE (selective 2'hydroxyl acylation analysed by primer extension), a technique that allows structural interrogation at each nucleotide, to further explore the structure of the FIV packaging signal RNA. Our analyses of wild-type RNA suggest that although the four conserved stem-loops are static structures, the 5' and 3' regions previously shown to form LRI also adopt an alternative, yet similarly conserved conformation, in which the putative DIS is occluded, and which may thus favour translational and splicing functions over encapsidation. Each conformer can be stabilised by mutation, and the LRI form in which the putative DIS is exposed forms dimers more efficiently in vitro. To further examine the role of SL5 in dimerisation, SHAPE and in vitro dimerisation assays were used to examine SL5 mutants. Dimerisation contacts appear to be made between palindromic loop sequences in SL5. As this stem-loop is located within the gag ORF, recognition of a dimeric RNA provides a possible mechanism for the specific packaging of genomic over spliced viral RNAs.

RETROVIRAL NC INTERACTIONS WITH SINGLE DNA OR RNA MOLECULES

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We use single molecule biophysical methods to quantify the DNA and RNA interactions of wild type and mutant human immunodeficiency virus type 1 (HIV-1) NC. We find that the nucleic acid interaction properties of HIV-1 NC can be altered significantly even after single point mutations. In particular, wild type HIV-1 NC shows rapid protein binding kinetics, significant duplex destabilization, and strong DNA aggregation, all properties that are believed to be critical components of nucleic acid chaperone activity. In contrast, mutation of any aromatic residue or specific groups of cationic residues, which disrupts virus infectivity, also inhibits NC's ability to facilitate DNA annealing. By stretching single DNA and RNA molecules using optical tweezers, we can precisely monitor the kinetics of NC's interactions. By measuring the force dependence of these kinetics, we are able to quantitatively characterize the structural dynamics of nucleic acids in the presence of wild type and mutant NC proteins. The results of complementary ensemble chaperone assays and cell-based studies of wild-type and mutant NC proteins will also be discussed.

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SINGLE MOLECULE DNA INTERACTIONS OF APOBEC3G

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Human APOBEC3G (A3G) is a cellular protein that inhibits reverse transcription and replication of human immunodeficiency virus type-1 (HIV-1) in the absence of the viral protein Vif. A3G binds single-stranded nucleic acids and impairs viral replication with a mechanism that is two-fold. First, A3G deaminates cytidine bases of viral single-stranded DNA (ssDNA). In a manner independent of this catalytic activity, A3G blocks minus-strand DNA synthesis by reverse transcriptase (RT), the viral DNA polymerase. Roughly 7 A3G proteins are packaged in each HIV-1 virion, requiring that each molecule rapidly locate multiple deamination sites on viral ssDNA, which is transient during reverse transcription. In contrast, blocking RT-catalyzed DNA elongation requires an extremely slow off-rate from single-stranded nucleic acids. It is possible that A3G exhibits fast kinetics as a dimer, allowing for rapid deamination activity, and slow kinetics as a mutimer, preventing RT from elongating viral DNA. We used single molecule DNA stretching to investigate these slow and fast binding modes. DNA force-extension curves in the presence of A3G exhibit approximately three times as much hysteresis, or disagreement between the extension and release curves, as those in the presence of the nucleic acid chaperone HIV-1 NC. This indicates that A3G binds ssDNA generated during extension and does not dissociate on the timescale of DNA release (approximately 30 seconds), preventing the two strands from reannealing. In contrast, pausing during DNA release at high extensions induces further A3G binding, and the DNA release curve strongly resembles that of ssDNA. Subsequent stretches reveal incremental A3G dissociation, which suggests that A3G multimerization on ssDNA is slow but reversible. These findings are consistent with a fast dimer binding mode for deamination and a slow multimer binding mode for inhibition of RT-catalyzed DNA extension.

CRYO-ELECTRON MICROSCOPY STUDIES OF HIV STRUCTURE

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BIOPHYSICAL ANALYSIS OF HTLV GAG TRAFFICKING AND PARTICLE MORPHOLOGY

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Much of the mechanistic details for how HTLV-1 Gag orchestrates virus particle assembly and release are poorly understood. Here, we monitored the behavior of both membrane-bound and cytoplasmic HTLV-1 Gag in real-time in living cells incubated on a fluorescence microscope. We used both fluorescence fluctuation spectroscopy (FFS, conventional and z-scan) and fluorescence imaging (epi-illumination, total internal reflection fluorescence (TIRF)) to investigate the relationship between cytoplasmic and membrane bound Gag, as well as particle release, using a Gag-YFP model system. FFS determines the brightness, mobility, and concentration (conventional) and localization (z-scan) of fluorescent particles from the intensity bursts generated by individual particles passing through a small observation volume, which yields information about protein stoichiometry, interactions, transport, and distribution. By coupling the single-molecule FFS technique with imaging techniques capable of monitoring Gag localization (epi-illumination) and membrane-specific localization (TIRF), we achieved new insights into the earliest events in HTLV-1 Gag assembly, and differences to HIV-1 Gag. We found that HTLV-1 Gag membrane-targeting occurred at all cytoplasmic concentrations measured, while appreciable membrane-targeting for HIV-1 required Gag cytoplasmic concentration to exceed a threshold. In addition, z-scan FFS revealed that a substantial population of membrane-bound HTLV-1 Gag exists not as puncta, but as a diffuse, low-order, dynamic “sheet.” These observations, coupled with previous observations of cytoplasmic Gag interactions and mobility, point to differences in membrane targeting of HTLV-1 and HIV-1 Gag. In summary, the use of biophysical fluorescence techniques, applied HTLV-1 Gag, has provided unparalleled information concerning HTLV-1 Gag trafficking processes in vivo as well as particle release, which has helped to identify assembly pathway differences between HTLV-1 and other retroviruses.

MECHANICAL PROPERTIES OF A SMALL VIRUS: A BIOLOGICAL PERSPECTIVE

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The advent of nanoscience and developments in atomic force microscopy (AFM) have recently led to the study of the mechanical properties of viruses. A previous comparison of the mechanical elasticity of the minute virus of mice (MVM) virion with those of its DNA-free capsid showed that the DNA molecule makes the virion anisotropically stiffer[1]. Increased stiffness is mediated by DNA segments bound to discrete sites at the capsid inner wall that exert a buttressing effect on the capsid structure[2]. Remarkably, the capsid regions around pores that are involved in a conformational rearrangement required for viral infection are free from bound DNA patches and remain mechanically elastic. These and other observations led us to formulate the hypothesis that local mechanical elasticity in a virus particle or other biomolecular complexes could be inextricably linked to a local propensity for reversible conformational rearrangements. We have provided experimental support for this hypothesis by using AFM to analyze the effect of many individual mutations on the mechanical elasticity of different regions in the capsid of the minute virus of mice. As predicted by the above hypothesis, any mutation that decreased elasticity at the regions around capsid pores also abolished the pore-associated, conformational rearrangement and virus infectivity; any mutation that had no effect on elasticity at those regions had no effect on the pore-related conformational change or virus infectivity. The available evidence provides strong support for a fundamental linkage in a virus particle between local elasticity and a capability for a biologically relevant conformational rearrangement.

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HIV-1 NUCLEOCAPSID COMPLEX: ARCHITECTURE AND PLASTICITY IMAGED BY ATOMIC FORCE MICROSCOPY

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Liberation of the RNA-associated nucleocapsid (NC) domains from the CA-MA layers is the first step in HIV-1 Gag polyprotein cleavage by the viral Proteases (PR). HIV-1 maturation then sees viral RNA condensation within the self-assembling capsid as PR shortens about 2000 RNA-bound NCp15 (132 aa) by two successive cleavages of their C-terminal domain, releasing successively NCp9 (71 aa) and the mature NCp7 (55 aa). This sequential processing of the NC-domain containing polypeptide correlates with the improvement of its nucleic acid chaperone character. Intrinsically linked, RNA condensation/aggregation and the chaperone activity of the accumulating NCp7 result in the establishment of a mature nucleocapsid complex competent for RT-catalyzed viral DNA synthesis(1). Several studies have shown that an essential step of this maturation may be the separation of the p6 moiety, as NCp15 and NCp9/NCp7 profoundly differ for their binding mode on single-stranded nucleic acids. We will present here how molecular reconstitution and Atomic Force Microscopy imaging at the level of single molecule/complex can directly show this process: ssDNA or/and RNA of several thousand nucleotides have been used as large scaffolds to load hundred of recombinant NCp15, NCp9 or NCp7 – and the architectural modifications of the complexes upon or without PR cleavage were analyzed by both EMSA, SDS-PAGE and AFM. A RT-driven synthesis of the NCp7-bound ssDNA scaffolds obtained in the context of NC binding and by direct cleavage of bound NCp15 by PR will also be presented, as well as topographic AFM images of the related complexes, showing for the first time a finely tuned tripartite reconstitution of HIV molecular machine, with some important insights in nucleocapsid complex architecture and plasticity during virus maturation.

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MODULATION OF HIV-1 PHYSICAL PROPERTIES BY THE PRESENCE OF PSI-RNA: AN ATOMIC FORCE MICROSCOPY STUDY

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We are interested in the physical characterization of HIV-1 Virus-Like Particles (VLP) and purified capsid cores towards a better understanding of HIV-1 assembly. Here we focus on the modulation of their pleomorphic properties, i.e. the various global shapes of VLP or cores observed within common environmental and biological conditions. Using an original combination of biochemical approach, Atomic Force Microscopy (AFM) imaging and sophisticated image analysis, we quantitatively characterized the pleomorphism observed for HIV-1 VLP, as well as for purified viral cores. Analyzing three-dimensional high resolution AFM images of un-fixed VLP and viral cores, we conducted a complete morphological characterization of mature and immature particles at statistic levels. In addition, we revealed that the presence of the HIV-1 Psi-RNA inside viral cores is decisive for HIV-1 morphology.

MONITORING HIV ASSEMBLY WITH SINGLE-MOLECULE SENSITIVITY

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HIV infection is spread through the virus's ability to package its genome into a protein capsid, surround it with a lipid bilayer, and transmit infectious particles to neighboring cells. HIV assembly is driven by the Gag polyprotein precursor that alone can assemble and release virus-like particles carrying a dimeric RNA genome. Previous work has provided us with structural snapshots of individual domains of HIV Gag, structural models of the RNA genome and its packaging signal, the structure of the hexameric capsid lattice, as well as images of intact immature and mature HIV virions. Live cell imaging using fluorescently tagged Gag has allowed direct visualization of HIV assembly in living cells. Here we establish the use of in vitro single-molecule fluorescence tehniques to monitor the dynamic events underlying the nucleation of HIV assembly. We have developed the technology and reagents needed to site-specifically label recombinant Gag and genomic RNA molecules with organic fluorophores. Fluorescently labeled Gag and genomic RNA elements, in the presence of excess unlabeled material, assemble into HIV virus-like particles in an *in vitro* assembly reaction that depends on the additional presence of phosphoinositols. We present here the initial results from fluorescence correlation spectroscopy measurements of the nucleation of HIV assembly.

COMPUTATIONAL METHODS FOR IDENTIFYING SMALL MOLECULE MODULATORS OF THE HIV-1 NCP7 ACTIVITY

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The HIV-1 nucleocapsid protein-7 (NCp7) is a highly basic, small zinc-binding protein that is being considered a new potential drug target for antiviral therapy. NMR solution structures that describe the interactions between NCp7 and both viral DNA and RNA are available, but suffer from some steric clashes and other problems that limited their use for structure-based ligand design. For the first time, we performed an in-depth quantum mechanics (DFT) and Molecular Dynamics (MD) study of the NMR-based complexes between the very flexible NCp7 protein and viral nucleic acids, in order to identify the molecular determinants responsible for the nucleotides interaction toward the protein surface. Results point out that the main inter-molecular interactions are of electrostatic nature and involve the many basic residues of the NCp7. An additional cluster of non-electrostatic contacts reinforces and stabilizes the adduct with nucleic acids. These MD-based structures are in agreement with a plethora of biological data and have been employed for the subsequent ligand design step. An High Throughput Virtual Screening was performed for a library of commercially available compounds. Representative frames of the MD were selected to derive structure-based pharmacophoric models, that were used as a query filter to screen the compounds library. A further docking step was performed to facilitate the selection of the most promising hits. The ten most active compounds identified in silico, also showing the highest chemical diversity among each others were submitted to biological assay. Two of them have shown a good inhibitory activity against the growth and replication of viral particles, in preliminary cell-based in vitro tests.

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RNAI-BASED THERAPY AGAINST HIV-1

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We propose a durable antiviral gene therapy by ex vivo transduction of CD34+ blood stem cells with a lentiviral vector that expresses antiviral short hairpins (shRNAs) that activate the RNAi pathway against HIV-1. Human T cells transduced with a single shRNA cassette resist HIV-1 infection, but the virus eventually escapes. We have documented diverse viral escape routes and have proposed different combinatorial strategies to prevent such escape. For instance, secondary shRNAs can be designed to block specific viral escape routes. The shRNAs can also be used in combination with regular antiretroviral drugs to block the development of resistant HIV-1 variants. Antiviral cassettes that express multiple anti-HIV shRNAs are currently being tested in the humanized immune system (HIS) mouse as pre-clinical animal model.

In an attempt to optimize the shRNA reagents we have reported that certain hairpin loops can improve the efficiency of any given shRNA inhibitor. Besides improved loop variants, we also came across a single loop variant that unexpectedly had lost most silencing activity. Even more intriguingly, we measured a gain of knockdown with a reporter that scores the activity of the passenger instead of the guide strand. The molecular determinants that drive this strand-switch were investigated by designing additional shRNA variants. Unusual RNA processing products were detected on Northern blot and identified by cloning and sequencing. A new mechanism for Dicer-independent shRNA processing will be revealed.

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INTERNATIONAL RETROVIRAL NUCLEOCAPSID SYMPOSIUM: PERSONAL REFLECTIONS

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The International Retroviral Nucleocapsid Symposium first held in Frederick, MD in June, 1998. This symposium was conceived to allow scientists from all over the world the opportunity to meet and discuss research activities on the truly remarkable protein, the retroviral nucleocapsid protein. Initially, the research activities and discussions were focused on the zinc fingers of the NC because of the high level of conservation of the CCHC motif, and its special chemistry. Subsequent symposia have underscored the multiple activities in the retroviral life cycle which are dependent on this protein and the current symposium continues to add new roles for the importance of the NC protein. We will present a brief overview of the research conducted on the NC protein and presented and discussed at the International Retroviral Nucleocapsid Symposia since 1998.

Abstracts Poster presentations

Poster Session 1: Monday, September 19 14:30 PM – 16:30 PM

CaixaForum Aula 2

Poster Session 2: Tuesday, September 20 17:30 PM – 19:30 PM

CaixaForum, Aula Laboratory

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VACCINE-NCP7 INHIBITOR COMBINATION DELAYS SHIV INFECTION AND CONTROLS VIRAL REPLICATION

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As women account for close to 50% of newly acquired HIV-1 infections worldwide, the development of an effective vaccine or topical compounds to prevent HIV transmission remains a major goal in the control of the AIDS pandemic. Although there has been some encouraging news on the development of an effective vaccine and a topical microbicide to prevent HIV-1 vaginal transmission, increasing emphasis is now placed on combining intervention strategies. Using a nonhuman primate model of heterosexual HIV-1 transmission, we tested whether a topical microbicide comprised of an inhibitor of the HIV nucleocapsid protein (1) that reduces viral infectivity can potentiate the efficacy of a partially effective T-cell-based HIV vaccine. A significant delay in simian/human immunodeficiency virus acquisition (Log-rank test; p=0.0416) was seen in vaccinated macaques that were repeatedly challenged in the presence of a microbicide comprised of a sub-optimal dose of an S-acyl-2-mercaptobenzamide thioester (SAMT) nucleocapsid zinc-finger inhibitor (ZFI). Peak acute viremia was lower (Mann-Whitney test; p=0.0387) and viral burden was reduced (Mann-Whitney test; p=0.0252) only in the combination-treated animals. Future experiments will investigate the effect of increased SAMT dosage in the microbicide and use of an improved vaccine. In addition, we will determine the mechanism of the protective effect conferred by the SAMT at the vaginal mucosa. These studies have implications for future HIV-1 prevention and development of prevention strategies employing topical microbicides/pre-exposure prophylaxis and HIV vaccines. (Reference 1: Miller Jenkins et al. 2010 Nat Chem Biol 6:887-9)

REDUCING THE NCP7-INDUCED VIRAL RNA PROTECTION BY A PHENYL-THIADIAZOLYLIDENE-AMINE DERIVATIVE EFFICIENTLY INACTIVATES HIV

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The viral nucleocapsid protein (NC) plays crucial roles in the HIV-1 virus replication, mainly through its nucleic acid chaperone properties [1]. These properties mostly rely on its highly conserved 'CCHC' zinc fingers. Due to its highly conserved structure, NC is a promising target for anti-HIV-1 therapy. Different classes of anti-NC molecules have already been developed. Zinc ejectors have been particularly investigated, and are now used as microbicides, to prevent viral transmission [[2]. Here, we report on the characterization of the inhibitory mechanism of phenyl-thiadiazolylidene-amine (WDO-217) that efficiently blocks the replication of HIV-$1_{IIIB,}$ HIV-2_{ROD} and SIV_{MAC} viruses. Virus particles pretreated with this small molecule were unable to infect permissive cells. Using the intrinsic fluorescence of Trp37 of NC(11-55), we found that WDO-217 efficiently removes zinc from the peptide and thus, leads to its unfolding. Zinc ejection was confirmed by the inability of the peptide in the presence of WDO-217 to destabilize the secondary structure of cTAR, the complementary sequence of the transactivation response element [3]. In addition, WDO-217 was also able to eject zinc from complexes of NC with nucleic acids, leading to a decrease in the viral RNA in cell free virions, likely as a consequence of a defective protection by the zinc-free NC protein.

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RELATIONSHIP BETWEEN INTRINSIC DYNAMICS OF NUCLEIC ACID AND ITS BINDING TO THE HIV-1 NUCLEOCAPSID PROTEIN

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The reverse transcription of HIV-1 consists in steps leading to the conversion of the single stranded RNA genome in a double stranded DNA. We are interested in the first stand transfer during which the neo-synthesized DNA migrates from the 5' end of genome to its 3' end. The TAR RNA hairpin and its complementary cTAR DNA hairpin are involved in the transfer. The association of these hairpins is facilitated by the HIV nucleocapsid protein (NC). NC is involved in the rearrangement of nucleic acids at several steps during reverse transcription. The protein binds to nucleic acids notably through its two-zinc finger domain that preferentially interact with unpaired guanines. NC(11-55) binding to the top half of cTAR (mini-cTAR), show the presence of a single binding site located in the lower stem despite the presence of several unpaired guanines. We probe the internal dynamics of mini-cTAR using ^{13}C NMR relaxation measurements. Relaxation times data have been analyzed by employing the model-free formalism and have been completed by dispersion relaxation experiments. Results established that several unpaired guanines are indeed involved in transient base pairing. The guanine bound by the C-terminal domain of NC(11-55) is characterized by its relative high mobility and by the fact that it is non involved in transient pairing. To get more insights into the cTAR DNA recognition by NC, we also investigated using NMR the interaction between NC(11-55), a truncated form of NC, and Emini-cTAR DNA (33 nt), an extended version of mini-cTAR. Results show that NC(11-55) appears able to recognized a unique site with the insertion of a guanine in the C-terminal zinc finger of NC(11-55). Interestingly the binding entails the loss of several imino protons resonances indicating a strong destabilization of the half part of the cTAR hairpin.

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UNDERSTANDING AND TARGETING PROTEIN-RNA INTERACTIONS WITHIN THE Ψ-PACKAGING DOMAIN OF HIV-1

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The Ψ-packaging domain of HIV-1 is used to specify for and control the packaging of, the genomic RNA into a budding immature viron.(1) Interference, through mutations and deletions, to the structure of the Ψ-packaging domain, severely diminish the ability of the virus to correctly package genomic RNA.(2) The Gag polypeptide is heavily involved in the binding, coating, trafficking and packaging of genomic RNA, with these processes believed to be initiated through the structural rearrangement of the Ψ-packaging domain, upon binding of Gag.(3) We investigated the interactions of HIV-1 viral proteins (Gag & NC), with RNA structural motifs within the Ψ-packaging domain of HIV-1 using a systematic incorporation of 2-aminopurine nucleotides. We performed a series of studies aimed at understanding the rearrangement of RNA, by the binding of both Gag and NC. We are also in the process of establishing a small molecule screen, using a fluorophore-quencher modified RNA, to identify compounds that can interfere with the RNA structural rearrangement caused by the binding of Gag. Small molecules that can interfere with the packaging of genomic RNA will be valuable tools in further understanding the role of the Ψ-packaging domain as well as validating the domain as a target for a more intense drug discovery program.

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INTEGRATION OF GLOBAL MEASURES OF POST-TRANSCRIPTIONAL REGULATION IN HIV INFECTION

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We have developed a cohesive approach to examining the dynamic cellular post-transcriptional environment, integrating three methods: RIP-chip, to specifically determine mRNA targets of RNA-binding protein, polysome profiling, to measure active translation, and 4-thiouridine stability profiling, to measure synthesis and decay rates of mRNAs. Using a global, integrated data set consisting of microarray analysis from each method, we can observe the effects of dynamic changes in RNA association with RNA-binding proteins on stability and translation in a unbiased manner. Investigating the targets of the RNA binding protein HuR in a Jurkat activation model, we show that mRNA targets that increase in association with HuR also increase in stability, while mRNA targets that decrease in association with HuR also decrease in stability. The same is true for translation as measured by polysome profiling. We apply this same paradigm to a T cell HIV infection model to investigate the post-transcriptional environment in early events in HIV infection. We investigate the role of HuR, as it binds the mRNAs of several post-entry cellular factors in HIV infection. We analyze global cellular synthesis and decay, including viral RNAs, at several time points following HIV infection, and report initial findings.

CHARACTERIZATION OF M-PMV CA-NC SPACER-LIKE DOMAIN

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Immature retroviral particles are assembled from Gag polyproteins, which in all retroviruses contain matrix (MA), capsid (CA), and nucleocapsid (NC) proteins. Previously, we identified the N-terminal sequence of Mason-Pfizer monkey virus (M-PMV) NC (NC15) as a critical element for the particle assembly. Secondary structure prediction of M-PMV CANC reveals a putative alpha helical structure comprising initial 13 amino acids of NC extended with 8 C-terminal amino acids of CA protein. This structural motif is similar to those described for spacer peptides in other retroviruses (Human immunodeficiency virus HIV, Rous sarcoma virus RSV, etc.). However, in contrast to HIV or RSV SP1, the M-PMV SP1-like domain is not released during the Gag processing. To determine the importance of individual residues within this sequence motif for the assembly of M-PMV and to determine the termini of SP1-like domain, we introduced a series of mutations in this region and studied their impact on assembly of M-PMV immature particles. The yield and shape of particles assembled *in vitro* form purified M-PMV CANC protein were analyzed by EM. Further biochemical analysis of synthetic peptides derived from the M-PMV CA-NC spacer-like domain was carried out.

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CONTRIBUTIONS OF THE RNA HELICASE DHH1 IN TY3 RETROTRANSPOSITION ASSESSED BY MUTATIONAL ANALYSIS

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Expression of the retrotransposon Ty3 occurs in the presence of alpha-factor pheromone in mating populations of *Saccharomyces cerevisiae*. Ty3 RNA is utilized as the template for translation of retroelement proteins and is also packaged into virus-like particles (VLPs). Like other retroelements, the untranslated region (UTR) of Ty3 mRNA is predicted to contain secondary structures comprising a packaging signal allowing for selective incorporation into VLPs. A body of evidence across various organisms suggests host RNA helicases are exploited to promote viral replication[1]. The 5' UTR of Ty3 is required for RNA packaging and formation of discrete Ty3 RNA cytoplasmic foci. These foci are hypothesized to be sites of VLP assembly, and colocalize with Ty3 protein and host proteins[2]. Several of these host proteins have been identified in cytoplasmic complexes known as P bodies and stress granules, and are implicated in mRNA maintenance and translation regulation[3]. Of particular interest is the RNA DEAD-box helicase Dhh1/RCK, a component of both P bodies and stress granules. In yeast, Dhh1 is associated with a complex that promotes transcription elongation, is a translational repressor and activator of mRNA decapping, and is required for Ty3 transposition. Deletion of *DHH1* decreased Ty3 RNA and protein levels when Ty3 was expressed under two different promoter systems. In the wildtype background, overexpression of GST-Dhh1 from a high copy plasmid had a dominant negative effect on Ty3 expression. Expression of GST-Dhh1 under the native *DHH1* promoter complemented the cellular growth defect of *dhh1*Δ but did not rescue Ty3 expression completely. Several conserved DEAD-box RNA helicase motifs have been shown to function in helicase/ATPase activity, RNA binding, and mRNP aggregation. A series of constructs have been designed to introduce mutations in conserved motifs within Dhh1 to better understand the contribution of Dhh1 function to Ty3 RNA stability and protein expression.

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IN VITRO INVESTIGATIONS OF THE IMPACT OF NUCLEIC ACIDS ON GAG PROCESSING BY HIV-1 PROTEASE

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HIV-1 Gag polyprotein directs the assembly of retroviral particles. During the late phase of infection, Gag is processed by the viral protease (PR) into the proteins necessary to gain an internal architecture absolutely required for viral infectivity, one of these being NCp7 that contains the fully processed RNA-binding domain. NCp7 is issued from PR-catalyzed processing of Gag to NCp15 thus to NCp9, which in turn allows the condensed assembly of viral RNA within the nucleocapsid. In conditions close to pH 7, *in vitro* cleavage efficiencies for NCp15 and NCp9 compared to the first Gag cleavage have been shown to be ten and hundred times less efficient respectively leading to a slow NC full processing. However, at pH 7, PR residual activity is reduced to less than 5% as shown with the classical enzymatic assay using a fluorogenic peptidic substrate, pH optimum lying between pH 4.5 and 5.

We investigated here the effect of nucleic acid molecules known to efficiently bind to NCp15, NCp9 and NCp7 (especially the M13 single-stranded DNA and an oligonucleotide that adopts a G-quadruplex combined with single stranded tails) using PR *in vitro* assays. We analyzed the cleavage of NCp7, NCp9, NCp15, CA-SP1-NCp7-p1-p6 proteins comparing direct electrophoresis and competitive fluorometric assays. We confirmed presence of nucleic acids to lead to a strong cleavage activation of NCp15 to NCp7. Our results clearly bring to light that cleavage of p1-p6 and NCp7-p1, but also of the primary site p2b-NCp7 are very strongly activated within the HIV-1 particle (a RNA-dependent mode of fast proteolysis) in pH ranges reaching physiological conditions (6-6.5 instead of 4.5-5).

All together our data leads to: i) a new model of protease activation, ii) an updating of Gag processing during HIV-1 maturation, iii) the design of new specific HIV maturation inhibitors.

PLATINATED NUCLEOBASES AS CHEMOTYPE FOR NUCLEOCAPSID INTERACTION

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Protonation, alkylation or coordination to a metal ion, such as Pt(II), Pd(II) or Au(III) of a nucleobase generally decreases the energy of its lowest unoccupied molecular orbital (LUMO), thereby improving the potential for π-stacking interactions with the highest occupied molecular orbital (HOMO) in aromatic aminoacids such as N-acetyl tryptophan. The finding from this laboratory that platinated (metallated) nucleobases enhance stacking interactions with tryptophan over the free nucleobase prompted extension to the tryptophan-containing zinc finger to understand the molecular basis of metal complex-zinc finger interactions.[1,2] The C-terminal knuckle of NCp7 (F2, Lys34 – Arg54) in the presence of $[Pt(dien)(9\text{-EtGua})]^{2+}$ (Pt-I, dien = diethylenetriamine, 9-EtGua = 9-ethylguanine)) and *trans*-$[PtCl(9\text{-EtGua})(pyr)_2]^{+}$ (Pt-II, pyr = pyridine), was studied in aqueous solution by a fluorescence quenching assay, NMR and Circular Dichroism (CD) spectroscopy and ESI-Mass Spectrometry (ESI-MS). Pt-I is *in principle* capable only of "non-covalent" interactions while Pt-II, with a substitution-labile chloride, is further capable of bond formation and the effects on protein with respect to conformation and zinc ejection may be studied. The ss hexanucleotide d(TACGCC), similar to that used previously was studied for comparison. Extension of these findings to the system $[MCl(dien)]^{n+}$ (M = Pd, Pt n = 2; M = Au, n =3) further showed covalent binding with zinc ejection with intermediate species being recognized through mass spectrometry.[3] These findings and others suggest that zinc finger proteins are excellent templates for metal ion exchange and ligand reactivity. This consideration has prompted study of the structures as new chemotypes for inhibition of NCp7 recognition and function. These studies will be summarized.

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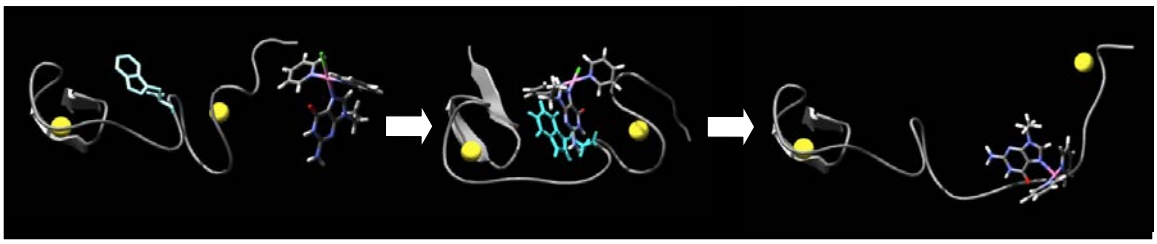

FROM TEST TUBE TO TISSUE CULTURE: TRANSLATING IN VITRO RESULTS WITH HIV NUCLEOCAPSID PROTEIN TO VIRAL FITNESS

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The HIV-1 nucleocapsid (NC) protein acts as a nucleic acid chaperone; it unravels RNA secondary structure, aggregates nucleic acids, and promotes genomic recombination. Crucial to NC's function are its two CCHC-type zinc fingers, which differ by five amino acids. The N-terminal finger contributes significantly more to NC's helix destabilizing activity than the C-terminal finger. In vitro analysis of mutant proteins in which single amino acids in the N-terminal zinc finger were substituted with residues from the C- terminal zinc finger revealed defects in helix destabilizing activity (I24Q, N27D) and nucleic acid binding (N27D) compared to wild-type NC. However, the growth rate of I24Q virus in tissue culture is comparable to wild-type virus, while N27D, which shows more severe chaperone defects in vitro, grew more slowly than wild type. These findings indicate that the helix destabilizing activity of NC may not be as critical to viral fitness in tissue culture and highlights the importance of correlating in vitro results to live viral fitness. Currently we are using cell culture based competition assays to test the relative fitness of NC mutants that show defective chaperone activity in vitro. The assays are designed to reveal even subtle differences in fitness. These experiments will help determine the extent of the correlation between in vitro chaperone activity and viral fitness.

CONFORMATIONAL CHANGES OF HIV-1 RNA UPON TRNA PRIMER ANNEALING STUDIED BY ENSEMBLE AND SINGLE-MOLECULE FRET TECHNIQUES

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During reverse transcription, HIV-1 uses cellular tRNA as a primer, which anneals to a complementary sequence in the viral RNA, the primer binding site (PBS). Additional interactions between the tRNA primer and viral RNA have found to play a role in reverse transcription. Of special interest was an 8 base motif, termed the primer activation signal (PAS), which interaction with tRNA we investigated through conformational changes of HIV-1 upon tRNA annealing. Specifically, the efficiency of conventional heat annealing methods was evaluated in comparison to using NC-mediated annealing. The methods employed were fluorescence resonance energy transfer (FRET) spectroscopy and single-molecule FRET microscopy. Our results demonstrate that the tRNA primer interacts also with the PAS motif, and that NC is more potent for forming compactly folded RNA structures compared to heat renaturation and annealing.

STRUCTURAL DETERMINANTS OF TAR RNA-DNA ANNEALING IN THE ABSENCE AND PRESENCE OF HIV-1 NUCLEOCAPSID PROTEIN

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Annealing of the TAR RNA hairpin to the cTAR DNA hairpin is an essential process in the minus-strand transfer step of HIV-1 reverse transcription. HIV-1 nucleocapsid protein (NC) plays a crucial role by facilitating annealing of the complementary hairpins. To gain insight into the mechanism of NC-mediated TAR RNA/DNA annealing, we used structural probes (nucleases and potassium permanganate), gel-shift annealing assays, gel retardation assays, fluorescence anisotropy and cTAR mutants under conditions allowing strand transfer. The data support a dynamic structure of the cTAR hairpin in the absence of NC, involving equilibrium between both the closed conformation and the partially open “Y” conformation. The apical and internal loops of cTAR are weak and strong binding sites for NC, respectively. NC slightly destabilizes the lower stem that is adjacent to the internal loop and slightly shifts the equilibrium toward the “Y” conformation. The annealing pathways in the absence of NC involve the fast formation of unstable kissing and zipper intermediates, followed by a rate-limiting strand exchange between the stems of hairpins. We show that the annealing intermediate of the kissing pathway is the loop-loop interaction involving 6 intermolecular base-pairs. Annealing via the zipper intermediate is slightly favored in the presence of NC.

DISRUPTION OF HIV-2 GENOMIC RNA DIMERISATION AFFECTS THE PROCESSING OF GAG AND IMPAIRS VIRAL INFECTIVITY

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The RNA packaging signal (Psi) of Human Immunodeficiency Virus type-2 (HIV-2) is located upstream of the splice donor and hence is present on both spliced and unspliced RNA. Encapsidation specificity is conferred by a co-translational mechanism and limited availability of the Gag protein. We have demonstrated previously that the viral RNA genome is dimeric within the virion particles and that motifs within the encapsidation signal are critical for this process. Our previous study has also shown that a mutation causing a reduction in genome dimerisation and packaging resulted in a decrease in the proportion of mature particles released. In the present study, we aimed at investigating the exact relationship between HIV-2 genome dimerisation, particle assembly and maturation and viral infectivity. To do so, we designed a series of viral mutants within Psi and the associated stem-loop 1 (SL-1) structure. Wild type (WT) and mutant viruses were evaluated for their ability to replicate in T-cells and evolution studies of the viruses were performed. The genomes of WT and mutant HIV-2 were analysed by native northern blot and their packaging efficiencies were determined. Finally, protein synthesis and processing was evaluated by pulse-chase metabolic labelling and immunoprecipitation. Our data confirm the requirement for a purine-rich sequence ($_{392}$-GGAG-$_{395}$) within Psi for efficient RNA dimerisation and viral replication and suggest that genome dimerisation is tightly linked to viral infectivity. Revertant viruses were obtained from long-term culture of dimerisation-deficient mutants and their analysis revealed a potential link between genome dimerisation and Gag processing, corroborating a previous study in HIV-1. Taken together, our results confirm the existence of a tight relationship between dimerisation of HIV-2 genomic RNA and viral infectivity, most likely through the correct processing of the Gag polyprotein and particle assembly.

HIV-1 NC protein modifies opening and closing kinetics of TAR RNA hairpin

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Retroviral nucleocapsid (NC) proteins are nucleic acid chaperones that play a key role in the viral life cycle, including reverse transcription, where NC destabilizes the transactivation response RNA (TAR RNA) hairpin. To quantify the interaction of HIV-1 NC and TAR RNA, we used optical tweezers to exert tension upon the free ends of the individual TAR hairpin, forcing the hairpin open and then allowing it to close. In the absence of NC, the TAR hairpin opens and closes at forces that depend upon the hairpin stability, as well as the rate of pulling and relaxation. Surprisingly, once saturated with NC, the opening forces increase, suggesting an apparent stabilization of the structure. However, the higher opening rate in the presence of NC is only observed at high pulling rates, while extrapolation of the measured TAR opening rate vs pulling rate to infinitely slow pulling yields ~1000-fold faster opening with NC. This ability of NC to facilitate opening of the TAR hairpin is equivalent to ~4 kcal/mol decrease in the TAR opening barrier. The stronger pulling rate dependence of TAR opening in the presence of NC indicates that fewer TAR base pairs unzip prior to complete hairpin stem destabilization. Specifically, of the 24 TAR hairpin stem base pairs it is sufficient to unzip 12 bp in the absence of but only 6 bp in the presence of NC to cause complete TAR unfolding. Furthermore, TAR unfolding measurements reveal that NC lowers the overall free energy of hairpin opening by ~3 kcal/mol. These results quantitatively characterize the ability of NC to moderately destabilize every nucleic acid base pair.

TRANSMISSION OF NEW CRF07_BC STRAINS WITH 7 AMINO ACID DELETION IN GAG P6

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A 7 amino acid deletion in Gag p6 (P6delta7) emerged in Chinese prevalent HIV-1 strain CRF07_BC from different epidemic regions. It is important to determine whether this mutation could be transmitted and spread. In this study, HIV-1 Gag sequences from 5 different epidemic regions in China were collected to trace the transmission linkage and to analyze genetic evolution of P6delta7 strains. The sequence analysis demonstrated that P6delta7 is a CRF07_BC specific deletion, different P6delta7 strains could be originated from different parental CRF07_BC recombinants in different epidemic regions, and the transmission of P6delta7 strain has occurred in IDU populations. This is for the first time to identify the transmission linkage for P6delta7 strains and serves as a wake-up call for further monitoring in the future; In addition, P6delta7 deletion may represent an evolutionary feature which might exert influence on the fitness of CRF07_BC strain.

Acknowledgments

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DEAMINASE AND BINDING ACTIVITIES OF APOBEC3A DICTATE ITS CELLULAR FUNCTION

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Human APOBEC3A (A3A) belongs to a family of enzymes displaying cytidine deaminase activity (conversion of deoxycytidine to deoxyuridine) on single-stranded (ss) DNA. It functions as a host cell defense factor by potently inhibiting retrotransposition of long interspersed-nucleotide element (LINE)-1, a class of mobile genetic elements that are potentially detrimental to the human genome, since they can insert into coding regions of functional genes. Retrotransposition of LINE-1 involves reverse transcription of its RNA to a double-stranded DNA. We set out to understand A3A's cellular function at a molecular level by carrying out cell-based LINE-1 retrotransposition assays with A3A mutants and correlating their levels of retrotransposition with catalytic activity. Overall, mutants defective in deamination were also found to exhibit less anti-retrotransposition activity. This suggests the role of A3A as a DNA mutator in carrying out its function against LINE-1. Like other members of the A3 family (e.g., A3G), A3A deaminates cytidines in a sequence-specific manner and this specificity has been shown to involve a certain flexible loop. Point mutations in the A3A loop as well as swapping this loop with the corresponding one in A3G totally eliminated cytidine deaminase activity and greatly abrogated inhibition of LINE-1 retrotransposition. Interestingly, for all A3A mutants tested, the effect of A3A on retrotransposition was dose-dependent. This suggests that increased A3A cellular expression allows it to regain activity against LINE-1, even in the absence of deaminase activity, which implies the additional existence of a deaminase-independent mechanism. To obtain further insights into the molecular properties of A3A, we used highly purified recombinant protein to study the kinetics of cytidine deamination and binding to nucleic acids. A3A bound ssRNA with much greater affinity than ssDNA, which is surprising as it displays no catalytic activity on ssRNA. Studies to probe the biological significance of A3A's biochemical activities are now underway.

THE BEHAVIOR OF THE HIV-1 NUCLEOCAPSID PROTEIN TOWARDS APTAMERS RAISED AGAINST HIV-1 PROTEIN TARGETS

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Currently, therapeutic management of HIV infection is generally based upon administration of combination therapy with multiple small-molecule drugs, an approach known as highly active antiretroviral therapy (HAART). However, the numerous challenges associated with the application of HAART, which include significant side effects, poor patient compliance, and the eventual emergence of escape mutants that ultimately render the drugs ineffectual in most cases, have prompted continued studies into alternative treatment strategies. In this regard, RNA aptamers are seen to show promise as potential drugs. In principle, these non-coding small nucleic acid molecules can disrupt key steps in the viral replication cycle by tightly binding to the targets against which they are raised. This binding in turn prevents the target from engaging in essential steps of the viral replication cycle, an effect that would be expected to ultimately curtail viral replication. Numerous aptamers against proteins essential to HIV-1 viral replication, including RT, IN, PR and NC have been developed. However, their efficacy in shutting down viral replication has in most cases been fairly modest. In our investigations of the interaction of various aptamers with NC, we have observed that these non-coding nucleic acids succumb to NC-mediated fragmentation. An implication of this finding is that the aptamers could be rendered non-effective *in vivo*, given the high intracellular concentrations of NC that are attained during viral replication.

NCP8 BINDS TO THE HIV-2 LEADER RNA DOMAINS INVOLVED IN THE LOOSE DIMER FORMATION

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Gag and Gag-derived nucleocapsid (NC) proteins are crucial for the HIV genomic RNA dimerization, packaging and virion assembly. Dimerization and packaging signals, closely linked in HIV-2, are located in the leader RNA region (5′ UTR) (1). We have identified motifs within the HIV-2 leader RNA that interact with the recombinant NCp8 protein (2). Our studies could be performed on shorter RNA, representing isolated motives of the HIV-2 leader RNA, because we have established that their structure remains the same within the 560 nt long leader RNA. Both SL1 and TAR as isolated domains are bound by recombinant NCp8 protein with high affinity, contrary to the hairpins downstream of SL1. Foot-printing of the SL1/NCp8 complex indicates that the major binding site maps to the SL1 upper stem.

The relatively tight binding of NCp8 to the TAR domain is especially interesting in view of the data indicating the involvement of the TAR hairpin III in the formation the HIV-2 leader RNA (+1–560) loose dimer (2). Two kissing loop interfaces within the loose dimer were identified: SL1/SL1 and TAR/TAR. Evidence for these findings is provided by RNA probing using SHAPE, chemical reagents, enzymes, non-denaturing PAGE mobility assays, antisense oligonucleotides hybridization and analysis of an RNA mutant. Taken together, these data suggest a model in which TAR hairpin III, the segment of SL1 proximal to the loop and the PAL palindromic sequence play specific roles in the initiation of HIV-2 RNA dimerization.

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DIFFERENTIAL ACTIVITY OF CHANDIPURA VIRUS NUCLEOCAPSID PROTEIN DEPENDING ON ITS OLIGOMERIZATION STATUS: FUNCTIONAL INSIGHTS

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A common feature of all Nucleocapsid proteins is their ability to self associate to form highly ordered oligomeric structures in association with the viral genomic material, known as the encapsidation complex. The mechanism underlying the specific recognition of the viral genome and its processive encapsidation is not entirely understood in most family of viruses. We have functionally characterised the Nucleocapsid protein, N of the Chandipura virus (CHPV), a lesser studied, human pathogenic, non-segmented negative strand RNA virus belonging to the Rhabdoviral family; with respect to its oligomerization status. Interestingly, it has been found that the N protein differentially binds RNA depending on its oligomeric state. It is the monomeric form that specifically recognizes the 3' non- coding termini of the viral genome (during initiation of encapsidation); whereas, upon oligomerization the RNA enwrapping becomes non-specific. The regions responsible for this differential RNA binding as well as for N-N self association have been delineated, using a battery of deletion mutants. The mechanism for maintaining N in its monomeric form has also been studied. It is found that another viral protein, the Phosphoprotein P serves this function, and exhibits N-specific chaperone activity, both *in-vitro* and *ex-vivo*. It has shown for the first time for any Rhabdovirus that, the monomeric N-P interacting domain overlaps the N-N self association domain. This explains how the N protein is kept in an encapsidation competent monomeric form during viral replication. On the other hand, for viral RNA synthesis, recruitment of the viral polymerase upon the N-RNA template is mediated through oligomer N-P interaction. Thus, N protein also interacts differentially with the P protein depending on its oligomerization status. The N domain responsible for this interaction has also been studied. Together, these observations provide a better understanding of the complex events that leads to encapsidation in this important human pathogen.

A ROLE FOR RAC1 AND RHOA GTPASES IN HIV-1 GAG ASSEMBLY AND RELEASE

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Our aim was to characterize the RhoGTPases and cell signaling pathways which are specifically modulated in T-cells during HIV-1 assembly and release. According to some studies, these steps involve membrane and actin cytoskeleton remodeling which is regulated by the RhoGTPases. In fact, some data suggests the implication of such proteins in HIV-1 release, and our preliminary results revealed that Rac1 and RhoA GTPases can modulate viral production, Gag membrane attachment and Gag localization in cells expressing HIV-1. Thus, Gag assembly and viral production might require a transitional regulation of cell signaling pathways mediated by RhoGTPases. The molecular mechanism underlying this effect is not yet understood and it raises the question of the role of these RhoGTPases in HIV-1 assembly?

We studied the implication of Rac1 and RhoA GTPases in virus assembly and release in Hela and T-cells. Theses RhoGTPases were either suppressed or overexpressed. For each condition, we analysed the intracellular Gag localization, viral assembly and production. Results showed in HeLa cells that Rac1 or RhoA inhibition by siRNA and the overexpression of Rac1 or RhoA dominant negative mutants can reduce viral production, modify intracellular Gag localization and reduce Gag membrane attachment. Moreover, the overexpression of Rac1 or RhoA WT and dominant positive mutants enhance viral production. In Jurkat T-cells expressing HIV-1, our preliminary results shown that siRNA inhibition of RhoA and Rac1 decrease viral production and modify Gag localization. We tested also the effect of Gag on the activation level of Rac1 and RhoA GTPases. Results shown that Gag can activate endogenous Rac1 and RhoA and increase the percentage of T-cells expressing F-actin.

Our results show that regulation of cell signalling by RhoGTPases is modulated during HIV-1 assembly and release in T-cells. We are further characterizing which are the cellular RhoGTPase mediated pathways regulated upon HIV-1 assembly and release.

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NOVEL ACTIVITY OF HIV-1 GAG ZINC FINGER MUTANTS: ABILITY TO PROMOTE MINUS-STRAND TRANSFER

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The NC domain in Gag determines Gag's ability to function as a nucleic acid chaperone. In a study of HIV-1 minus-strand transfer *in vitro*[1], we found that high concentrations of Gag drastically inhibit the DNA elongation step by a "roadblock" mechanism. This result is consistent with "nucleic acid-driven multimerization" of Gag reflecting CA-CA interactions and the slow dissociation of Gag from bound nucleic acid, which could potentially prevent premature initiation of reverse transcription. To investigate the role of Gag's zinc fingers (ZF) in chaperone activity, we tested ZF mutants (H400C, H421C, H400/421C, ΔZF1, ΔZF2, and ΔZF1+2) in our reconstituted minus-strand transfer system with model (-) strong-stop DNA and acceptor RNA substrates. At saturating concentrations, these mutants behave like WT Gag in "annealing only" assays, although there is some variation at lower concentrations. This observation suggests that the ZF mutations do not have a significant effect on aggregation and binding to these nucleic acids[2]. In assays of annealing plus DNA elongation, the effects are concentration-dependent. At a low concentration (0.12 μM), all of the mutants (except H421C) have reduced activity (~2-fold lower than WT Gag). Surprisingly, when the concentration is raised to 0.23 μM, WT Gag activity is decreased, while the activity of all of the mutants is increased. As the protein concentration is further increased, the strand transfer activity of the mutants is also decreased, indicating that the ZF mutants are subject to the roadblock mechanism, but at a 2- to 4-fold higher concentration than is seen with WT. Based on these results, we speculate that the ZF mutations raise the threshold for the roadblock inhibition due to slightly reduced nucleic acid binding affinity and/or possible protein conformational changes. Other experiments are in progress to assess the effect of the mutations on $tRNA^{Lys3}$ elongation during initiation of reverse transcription.

1. Wu T, Datta SAK, Mitra M, Gorelick RJ, Rein A, and Levin JG. (2010) Virology **405**:556-567.
2. See abstract by C.P. Jones et al.

IDENTIFICATION OF A SPECIFIC PROTEIN INTERACTION BETWEEN HIV-NC AND YY1 AND ITS EFFECT ON THE VIRAL PRODUCTION

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HIV nucleocapsid protein (NC) is a small, highly basic, nucleic acid binding proteins containing two zinc-finger motifs. Many studies showed that the NC protein participates constantly in every step during virus production. To further understand the undisclosed role of NC protein, we screened host cell factors interacting with NC protein through in vitro pull-down assay and yeast two-hybrid assay. Among them, a transcription factor Yin Yang 1 (YY1), which is known previously as a negative regulator of HIV-1 LTR expression leading to decrease of virus production when over-expressed, is identified as one of NC binding protein. The specificity of interaction between NC and YY1 was further confirmed by co-immunoprecipitation experiments using deletion mutants of YY1 protein and zinc finger mutants of NC protein in mammalian cells. To determine the role of YY1 and importance the interaction with NC protein in the virus life cycle, over-expression and knock-down experiment of YY1 were first carried out in 293FT cell in conjunction with transfection of pNL4-3GFP proviral DNA. The results show that mRNA levels of HIV were decreased in a dose-dependent manner when YY1 was over-expressed while a reverse was seen in knock-down condition of YY1. However interestingly, the p24 ELISA and western blot results reveal that HIV production was decreased when YY1 was knocked down although the level of HIV transcripts was increased. A similar result was observed with using a HIV-LTR driven reporter plasmid, which showed that the expression level of reporter gene protein is increased consistent with mRNA level. We reason that these results alltogether could suggest that YY1 might participate in post-trasnscriptional and/or translation step of HIV life cycle. The effect of NC on these interesting phenomena is currently investigating and will be presented in the meeting in detail.

HIGHLY CONSERVED SERINE RESIDUE 40 IN HIV-1 P6 REGULATES CAPSID PROCESSING AND VIRUS CORE ASSEMBLY

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The HIV-1 p6 Gag protein regulates the final abscission step of assembling virions from the plasma membrane by the action of two late assembly (L-) domains. Although p6 is one of the most polymorphic regions of the HIV-1 *gag* gene, most functional domains overlap with several conserved residues in p6. In this study, we investigated the importance of the highly conserved serine residue in position 40, which until now was not assigned to any known function of p6.
Consistently with previous data, we found that mutation of Ser-40 has no effect on ALIX mediated rescue of HIV-1 L-domain mutants. However, the only feasible S40F mutation that preserves the overlapping *pol* open reading frame (ORF) reduces virus replication in T-cell lines and in human lymphocyte tissue cultivated *ex vivo*. Most intriguingly, L-domain mediated virus release is not dependent on the integrity of Ser-40. However, the S40F mutation significantly reduces the specific infectivity of released virions. Further, we observed that mutation of Ser-40 selectively interferes with the cleavage between capsid (CA) and the spacer peptide SP1 in Gag, without affecting cleavage of other Gag products. This deficiency in processing of CA in consequence led to an irregular morphology of the virus core and the formation of an electron dense extra core structure. Moreover, the defects induced by the S40F mutation in p6 can be rescued by the A1V mutation in SP1 that generally enhances processing of the CA-SP1 cleavage site.
Taken together, we found that, although located in close proximity to the ALIX binding site, the mutation of Ser-40 does not affect ALIX-mediated HIV-1 budding, but selectively inhibits CA maturation, viral core formation, and infectivity.

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