

Meeting report

Viruses in and out

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Published: 24 September 2002

Genome **Biology** 2002, **3(10)**:reports4033.1–4033.4

The electronic version of this article is the complete one and can be found online at <http://genomebiology.com/2002/3/10/reports/4033>

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A report on the twelfth Congress of Virology, part of 'The world of microbes', the joint meeting of the three divisions of the International Union of Microbiological Societies, Paris, France, 27 July to 1 August 2002.

Microbes are the smallest forms of life on earth. Some microbes are deadly, most are harmless, and some are extremely beneficial. They can be found anywhere - in air, water, plants, animals and humans - and fall into four major categories: fungi, protozoa, bacteria (including Archaea, in this context), and viruses, which are the smallest of all. The international meeting 'The world of microbes' was divided into congresses on mycology (on fungi), bacteriology and applied microbiology, and virology; I will focus on the latter here.

Viruses are replicating microorganisms that are heavily dependent on the structural and metabolic components of the host cell. Viruses can infect bacteria, fungi, plants, invertebrates and vertebrates. Whatever the host, virus particles (or virions) must penetrate the cell and uncoat their structure to allow transcription and translation of their genomes by the viral and the host machinery. Once the viruses have replicated, new virions are released from the infected cells. Even though most viral infections result in no symptoms, many viruses can cause virulent disorders, such as acquired immune-deficiency syndrome (AIDS), hemorrhagic fever, yellow fever, rabies or poliomyelitis. Viruses are classified in different taxonomic groups on the basis of their structural, physicochemical and replicative characteristics, and the meeting sessions were organized along these lines; I will focus on the sessions on the movement of plant viruses and the structure, assembly and entry of some enveloped viruses that infect vertebrates.

Movement of plant viruses

In contrast to animal viruses, which penetrate the cell after specific binding of a virion protein to a receptor on the cell

surface, plant viruses enter cells in the first instance by passive diffusion through breaches in the cell wall. This is later followed by spreading of the virus from cell to cell in the plant through plasmodesmata, cytoplasmic connections through channels in the cell wall that provide communication between adjacent cells. The viruses can then spread through the conductive tissues of the plant - xylem and phloem vessels. Susan Angell (John Innes Centre, Norwich, UK) presented new data on the movement of potato virus X, a plant virus with a single-stranded RNA genome. Four viral proteins are required for cell-to-cell movement: the triple gene block proteins (TGB 25K, TGB 12K and TGB 8K) and the coat protein. TGB 12K increases the size exclusion limit (SEL) of plasmodesmata, so that viruses can get through. Angell identified host plant proteins that interact with viral movement proteins, using a yeast two-hybrid system with the TGB 12K protein as a bait. Three plant proteins were identified, called TIP1, TIP2 and TIP3, which specifically interact with TGB 12K but not with TGB 25K or TGB 8K. Silencing the genes encoding these TIP proteins seriously perturbed cell-to-cell movement of potato virus X. Angell proposed a model in which β -1,3-glucanase, a key regulator of the plasmodesmata SEL, probably interacts with TIPs but not with TGB 12K and increases the SEL of plasmodesmata to allow virus movement.

Several talks were given on the movement of the tobacco mosaic virus (TMV). Elizabeth Waigmann (University of Vienna, Austria) presented new data on a novel plant protein called MPB2C that interacts with tobacco mosaic virus movement protein (TMV-MP). MPB2C is homologous to the plant myosins, and it interacts with movement protein *in vivo* and biochemically *in vitro*. It contains a hydrophobic region and a coiled-coil region, features that characterize cytoskeleton-associated factors. MPB2C was found to specifically block movement of TMV from cell to cell by anchoring TMV-MP to the cytoplasmic bodies (lipid droplets) or microtubules within the cell, preventing the virus from being targeted to the plasmodesmata. Vitaly Boyko (Friedrich Miescher Institute, Basel, Switzerland) presented very informative movies

made in order to investigate the association of TMV-MP with microtubules and the role of microtubules in transport of TMV RNA. The movies, achieved by expressing mutant or chimeric TMV-MP proteins fused to the green fluorescent protein (GFP), confirm that microtubules are important in cell-to-cell transport of TMV RNA.

Enveloped viruses infecting vertebrates

Several talks presented new data on measles virus, a member of the paramyxoviridae, with a negative-stranded RNA genome. Roberto Cattaneo (Mayo Clinic, Rochester, USA) presented very interesting data on measles virus particles, which are extremely variable in size, ranging from 120 nm to 300 nm in diameter. Given that the envelope is about 20 nm thick, Cattaneo and colleagues estimated that the cargo space of the particles - the total space contained within the envelope - may vary from $3 \times 10^5 \text{ nm}^3$ to over 10^7 nm^3 .

Cattaneo also presented evidence that measles virus particles have a hexameric genome length: that is, it is constrained to be a multiple of six nucleotides long, as has been shown previously for the model paramyxovirus Sendai virus. Hexameric virus genomes are often associated with RNA editing, which occurs through 'stuttering' of the RNA polymerase at specific sites; this is also true for measles virus. Cattaneo showed that the measles virus genome is hexameric using a chimeric virus encoding two CD4 domains appended to the hemagglutinin protein, which disrupt the protein's function in supporting envelope fusion. It appeared that the modified genome either acquired stop codons or gained an A or a G residue through RNA editing at an A_6G_4 sequence in the first CD4 domain. The insertion led to a reading-frame shift and therefore interrupted translation of the appended domain; hexameric genome length was restored through deletion of an A at an A_5G_4 sequence at the beginning of the polymerase large subunit reading frame, leading to a truncated polymerase. This demonstrates that not only the known editing site in the phosphoprotein gene but also polypurine runs are prone to polymerase stuttering. Cattaneo and colleagues also showed nicely that measles virus becomes polyploid after coinfection with measles viruses modified by addition of genes encoding fluorescent proteins; after multiple passages, a significant fraction of progeny syncytia co-express both fluorescent proteins, showing that several genomes are present in one syncytium.

The matrix protein of measles virus, which is found associated with the virion envelope, has a key role in virus assembly because it mediates contact between the nucleocapsid proteins, the main internal structural proteins, and the viral glycoprotein complexes at the surface of infected cells. Andrea Maisner (Institut für Virologie, Marburg, Germany) and co-workers generated a recombinant virus lacking most of the cytosolic portion of the two glycoproteins, hemagglutinin and

fusion proteins. This allowed them to build a model of the assembly of measles virus, in which the matrix protein, which binds to the cytoplasmic tails of both glycoproteins, orchestrates their positions at the budding site, thereby preventing endocytosis of the glycoproteins and minimizing cell-to-cell fusion. In doing so, the matrix protein determines the protein composition and cytopathic properties of the virus. Anthony Schmitt (Northwestern University, Evanston, Illinois, USA) got similar results in a paramyxovirus, simian virus 5 (SV5). Unlike the situation in some negative-stranded RNA viruses, such as vesicular stomatitis virus and ebola virus, it was found that SV5 matrix protein alone cannot induce vesicle budding and is not secreted from cells; rather, it needs one of the two glycoproteins (hemagglutinin or fusion protein) in the presence of the nucleocapsid protein. The cytoplasmic tails of the two glycoproteins also seem to be very important for SV5 assembly.

In the session on the molecular biology of human immunodeficiency virus (HIV), Xavier van Ostade (University of Gent, Belgium) presented a new system designed to trap mammalian protein-protein interactions, called MAPPIT (Figure 1). As mentioned earlier, viruses are highly dependent on the host machinery for their propagation. Understanding how a virus can direct a cell to sustain its needs, presumably through interactions between viral and cellular proteins, is therefore essential. By contrast with the yeast two-hybrid system, the MAPPIT approach detects protein-protein interactions in their normal physiological context and is especially applicable to the *in situ* analysis of signal-transduction pathways. In addition, it allows the detection of both phosphorylation-dependent and phosphorylation-independent interactions in intact human cells. Van Ostade and co-workers showed that this method can be used to screen complex cDNA libraries, and they identified new interaction partners in the activation pathway of the erythropoietin receptor.

An entire session was dedicated to retroviruses. In the first talk, Volker Vogt (Cornell University, Ithaca, USA) presented some new data on Rous sarcoma virus, which requires full-length Gag protein and nucleic acid, either RNA or DNA, for its assembly. Vogt and colleagues were able to show, using oligonucleotides with simple GT repeats, that the minimal length of nucleic acid required to support efficient assembly *in vitro* is 16 nucleotides; this is exactly twice the size of the nucleotide-binding site of Gag on DNA (8 nucleotides). They therefore proposed a model in which nucleic acid promotes the formation of Gag dimers, which are critical intermediates for viral assembly (Figure 2). Replacement of the nucleocapsid domain of Gag, which is essential for assembly of Gag into viral capsids, with a leucine zipper led to the formation of virus-like particles, and insertion of a short flexible linker upstream of the leucine-zipper motif or of the nucleocapsid domain led to budding of tubular rather than spherical particles. In the

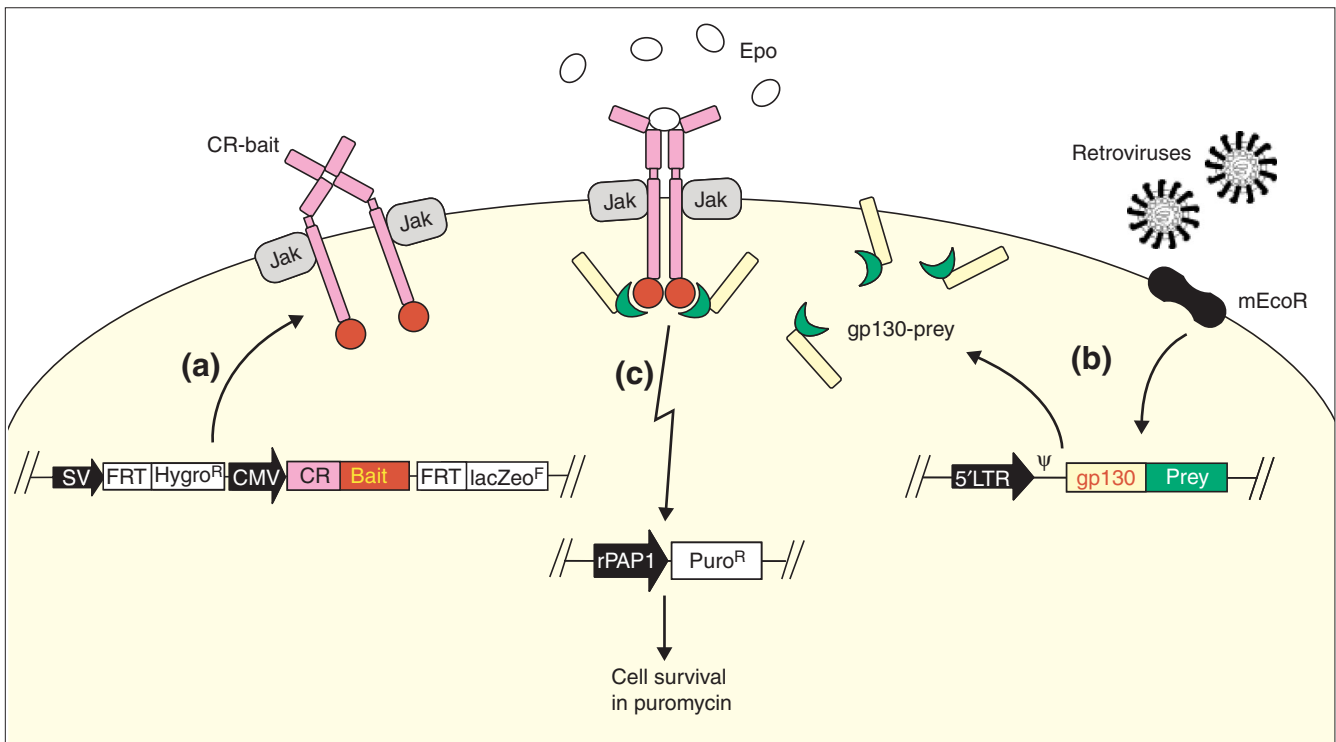


Figure 1

A schematic diagram of the mammalian protein-protein interaction trap, MAPPIT. The selection system makes use of a particular HEK293 cell clone that has a Flp recombination target (FRT) integration cassette in a transcriptionally active locus. These cells also stably express the murine ecotropic retroviral receptor (mEcoR) and have a stably integrated puromycin resistance gene. **(a)** Cells expressing the chimeric type I erythropoietin (Epo) receptor fused to the bait at the carboxyl terminus (CR-bait) are generated by Flp recombinase-assisted integration followed by hygromycin selection. **(b)** Hygromycin-resistant cells are subsequently infected for 24-48 hours with a retrovirus encoding gp130 (part of the signaling cascade) fused to the prey protein (gp130-prey). **(c)** Cells are treated with erythropoietin for another 24-48 hours before puromycin selection. If the bait and prey proteins interact, binding of the ligand to the receptor can induce a signaling cascade through the Janus kinase (Jak), leading to the induction of the puromycin-resistance marker (Puro^R) and thus to growth of the cells in puromycin-containing selective medium. Prey proteins can be rapidly identified by direct amplification of their transcripts from lysed cell colonies by reverse-transcriptase PCR. Adapted with permission from Eyckerman S, Verhee A, der Heyden JV, Lemmens I, Ostade XV, Vandekerckhove J, Tavernier J: *Nat Cell Biol* 2001, **3**:1114-1119.

second talk, Axel Rethwilm (Technische Universität Dresden, Germany) proposed a model for the assembly of foamy viruses, in which viral RNA serves as a bridging molecule between Pol and Gag protein molecules to assemble a functionally active foamy virus capsid.

To close the session, two talks were given on porcine endogenous retroviruses (PERVs), which can cause problems for prospects of transplantation of pig organs into humans (xenotransplantation). Three infectious families of PERV have been identified: PERV-A and PERV-B can infect human

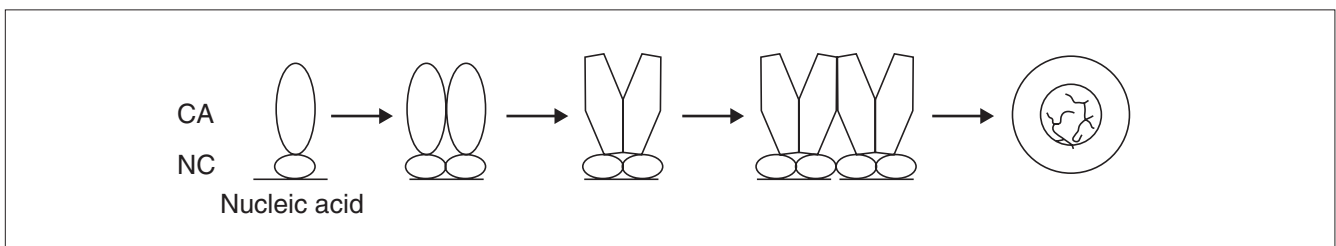


Figure 2

A model of the dimerization of Rous sarcoma virus Gag protein, as proposed by Volker Vogt. Only two domains of Gag are shown (nucleocapsid, NC and capsid, CA). Upon binding to a nucleic acid, two adjacent Gag protein molecules undergo a conformational change, which exposes a surface that can mediate dimer-dimer interaction. The Gag dimers are used as building blocks for assembly of the virus particle. Adapted with permission from Ma YM, Vogt VM: *J Virol* 2002, **76**:5452-5462.

cells *in vitro*, whereas PERV-C can infect only porcine cells. Clive Patience and co-workers (Immerge Bio Therapeutics, Charlestown, USA, and University College London, UK) reported the identification of two human receptors for PERV-A. The natural function of these receptors is unknown; a baboon homolog of the human receptors was active in human cells, whereas the murine homolog was not. The expression of the two human receptors for PERV-A was found to be widespread in the human tissue. Finally, Linda Scobie (University of Glasgow, UK, and Immerge Bio Therapeutics, Charlestown, USA) presented data on mapping and analysis of PERVs in the genomes of two strains of pig, 'Large White' pigs and miniature swine. In the Large White sow, PERV-A appeared to be present only in limited numbers of defective elements; some copies of PERV-B were identified, and no copies of PERV-C were present. The prevalence of PERV-B loci identified was found to be polymorphic within the more distantly related 'Large White hDAF' pigs. Scobie and colleagues applied this mapping strategy to analyze a particular line of miniature swine that does not appear to possess transmissible PERVs. The lack of intact proviral genomes was confirmed, and the strategy is currently being repeated for a strain of miniature swine that can transmit human replication-competent PERVs.

In both plants and animals, better knowledge of the mechanisms by which viruses replicate and interact with infected host cells is critical for the development of antiviral therapies and/or vaccines. As detailed by Kenneth Berns (Mount Sinai School of Medicine, New York, USA) during a session on the pathogenesis of viral infection, vaccines against HIV, hepatitis C, human papillomavirus, cytomegalovirus and herpes simplex virus are still needed. Alongside antiviral drugs and live and attenuated vaccines, new approaches such as RNA interference, which consists of silencing genes using sequence-specific double-stranded RNA, are promising for the prospects of antiviral therapy.