Cells and Viruses / HIV

S11-01

"Mx proteins: role in host defence against RNA viruses"

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Viruses of the *Bunyaviridae* family cause a variety of diseases ranging from uncomplicated fever to potentially lethal encephalitis and hemorrhagic fever. Little is still known about the factors determining pathogenicity in the vertebrate host. The interferon-induced MxA protein encoded on human chromosome 21 is a dynamin-like GTPase with antiviral activity against several negative strand RNA viruses, notably influenza viruses. Here we show that MxA inhibits representative members of the *Bunyaviridae* family (including Hantaan virus and Rift Valley fever virus) by interacting with an early step of virus replication. Mutant MxA-T103A carries a Thr to Ala substitution at position 103 within the GTP binding consensus sequence, does not bind GTP, and has no antiviral activity. When coexpressed with wild-type MxA in 3T3 cells, this inactive mutant protein suppressed the antiviral action of MxA. The interfering MxA mutant may disturb the formation of active wild-type MxA oligomers. Alternatively, it may prevent the proper folding of wild-type MxA monomers or compete with the wild-type protein for a viral target.

S11-02

Receptor length determines measles virus entry efficiency

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The pH-independent fusion of membranes induced by measles virus (MV) requires besides the fusion-competent protein F also the hemagglutinin (H) and, on the target membrane, the viral receptor CD46. We constructed chimeric receptors composed of different numbers and combinations of the four CD46 short consensus repeat (SCR) domains followed by immunglobulin-like domains of another cell surface protein, CD4. Chimeric proteins containing SCR I and II bound MV particles and conferred fusion function to rodent cells. SCR III-IV reinforced binding. Increasing the distance between the MV binding site and the transmembrane domain enhanced virus binding but reduced fusion efficiency. A hybrid protein about 120 Å longer than the standard receptor lost fusion-mediating function and was dominant negative over a functional receptor, indicating that several receptor molecules are necessary for MV-induced fusion. We postulate that efficient membrane fusion relies on a scaffold formed by interacting CD46 and H proteins. Receptor protein length determines the scaffold height and thus fusion efficiency.

S11-03

NUCLEAR TRAFFICKING OF ADENOVIRUS AND INFLUENZA VIRUS

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Signal-mediated nuclear import of proteins requires at least three steps, nuclear localization sequence (NLS) binding to a specific receptor, docking of the import substrate to the pore complex and hydrolysis of nucleoside triphosphates by the transport machinery. Protein import into the nucleus through the nuclear pore complex can be inhibited by reagents like wheat germ agglutinin (WGA) or the RL1 antibody which bind to cytosolic pore complex components. Alterations of lumenal pore complex domains, e.g., depletion of calcium from the nuclear envelope/endoplasmic reticulum, also block import of NLS-containing proteins and diffusion of small dextrans into the nucleus of higher eucaryotic cells. We tested if nuclear transport of two large macromolecules, the adenovirus deoxyribonucleoproteins (vDNP's), and the influenza virus ribonucleoproteins (vRNP's), would be affected under protein import-inhibiting conditions. Nuclear import of both genomes was severely obstructed by microinjection of WGA or RL1. WGA and RL1 prevented docking of incoming adenovirus at the nuclear envelope and inhibited virus disassembly. In lumenal calcium-depleted cells, vDNP and vRNP import was arrested, but adenovirus binding to the nuclear envelope and disassembly were not affected. In turn, nuclear import of newly synthesized influenza virus M1 protein, a key regulator for nuclear export of newly synthesized vRNP's, was blocked and vRNP export to the cytoplasm quantitatively repressed. The results suggest that adenovirus and influenza virus use existing cellular pathways controlled by the nuclear pore complex for the introduction of their genomes into the nucleus.

S11-04

Replication Strategy of Foamy Viruses

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Foamy viruses (FVs) make up a separate subgroup of retroviruses. While there is no evidence that FV are of medical importance, the study of the molecular biology disclosed some interesting features of the FV replication cycle which set these viruses apart from the other retroviruses. Some of these functional differences include the method of packaging the genomic RNA, the mechanism of Pol expression, aspects of regulating FV gene expression, and the presence of DI genomes in vitro and in vivo.
Most interestingly, recent investigations on the replications strategy of FV indicate that they share many features with the hepadnavirus group suggesting that the FVs may represent a functional link between the retroviruses and the pararetroviruses.

S11-05

THE NONSTRUCTURAL C PROTEIN OF MEASLES VIRUS IS NOT ESSENTIAL FOR MULTIPLICATION IN CELL LINES

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A reverse genetics system developed for measles virus (MV) allows now to manipulate the genome of this negative-strand RNA virus at the level of DNA. To obtain MV, a cell line (293-3-46) stably expressing T7 RNA polymerase and MV nucleocapsid (N) and phosphoprotein (P) protein is transfected with two plasmids. From one plasmid, T7 RNA polymerase produces MV antigenomes which are immediately encapsidated by N and P. From the other, mRNA is made encoding the MV L protein. The assembly of RNP (antigenome associated with N, P and L) starts MV replication. During normal infection, two nonstructural proteins (V, C) are produced. Both are encoded by reading frames partially overlapping that encoding P. C is translated from P mRNAs starting at the second AUG. Applying the new system, we investigated whether C is essential for multiplication. The C reading frame was silenced without changing the amino acids of P. Indeed, viable MV was generated. Its multiplication in Vero cells appeared indistinguishable from standard MV. The plaque size was slightly reduced to ~65%.

S11-06

PROTEIN INTERACTIONS OCCURRING DURING THE ASSEMBLY OF THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)

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Type-C retroviruses and lentiviruses like HIV-1 assemble at the cytoplasmic face of the cell membrane. Expression of the internal structural proteins, the so-called gag proteins of those viruses is sufficient for the formation of viral particles. Incorporation of the viral envelope glycoproteins into such particles, however, is necessary to render them infectious.

The viral envelope glycoproteins are directed to the site of assembly through the secretory pathway of the host cell while the gag polyprotein precursors are synthesized in the cytoplasm and then transported to the plasma membrane. Little is known about the mechanisms that establish the co-localisation of the different viral structural components at the cell membrane.

Using transient expression from subviral constructs as well as *in vitro* expression of the viral structural proteins we are trying to define functions on those proteins which drive viral particle formation. We are particularly interested to learn whether information contained in the long (150 amino acids) cytoplasmic tail of the gp41 subunit of the envelope glycoprotein is responsible for the specific uptake of the viral envelope into the budding particles.

S11-07

Prion propagation in PrP null mice with ectopic PrP expression

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The agent causing scrapie and other neurodegenerative diseases in humans and animals, the prion, is thought to be congruent with PrP^{Sc}, an abnormal isoform of the cellular prion protein (PrP^{C}). Prion propagation occurs mainly in brain and to a lesser extent in spleen. Mice with disrupted PrP genes are resistant to scrapie, and do not propagate prions.

To determine whether PrP^{C} expression was the only prerequisite for propagation of prions, PrP-deficient mice were reconstituted with transgenes encoding PrP driven by heterologous promoters. Scrapie-inoculated mice transgenic for a PrP gene under the control of the lck promoter overexpressed PrP on the surface of T-cells but surprisingly did not propagate prions in either thymus or spleen. Using an IRF-1 promoter/Eμ enhancer to drive PrP expression we generated mice with high levels of PrP on B-cells, T-cells and dendritic cells and low levels in the brain. Six months after inoculation with scrapie prions these mice accumulated ~10^7 LD_{50} units of scrapie infectivity in the spleen but had no infectivity in circulating leucocytes and in the brain. These findings show that overexpression of PrP does not suffice for prion replication and suggests that prion replication in the spleen involves cells other than B or T lymphocytes; dendritic cells are possible host cells.

S11-08

Replication protein A and HIV-1 nucleocapsid protein p7 influence strand displacement DNA synthesis of HIV-1 and FIV reverse transcriptases

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We have recently described the strand displacement DNA synthesis activity of the reverse transcriptases (RT) from HIV-1 (Hottiger et al., J. Biol. Chem. 269, 966-991, 1994) and FIV (Amacker et al., J. Virol. 69, 6273-6279, 1995). In search for factors influencing this particular RT activity, we found a cellular as well as a viral protein, being human replication protein A (RP-A) and the HIV-1 nucleocapsid protein p7 (NCp7). The single-stranded DNA-binding protein RP-A stimulated the strand displacement DNA synthesis by the lentiviral RTs in a concentration-dependent manner. On the other side, NCp7 inhibited strand displacement DNA synthesis and in higher concentrations even abolished DNA-dependent DNA synthesis by RT on a double-stranded DNA substrate with a defined gap of 26 nucleotides.

S11-09

Apoptosis and membrane g-glutamyltransferase (g-GT) in peripheral blood mononuclear cells from HIV patients.

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Peripheral blood mononuclear cells (PBMN) isolated from 161 HIV-positive patients and from 29 healthy donors were examined for conventional immunologic markers (CD3, CD4, CD8, CD19, CD14). The clinical stage of 45 patients was known: 58% A (asymptomatic individuals), 18% B (opportunistic infections) and 24% C (overt AIDS). Additional parameters were measured on PBMN cells: percentage (%) of apoptosis by flow cytometry of the propidium iodide uptake (permeability test) and the specific activity of g-glutamyltransferase (g-GT), an ectoenzyme contributing to the intracellular transfer of glutamyl residues for the synthesis of the antioxidant glutathione (GSH), and which has been previously shown to be involved in apoptosis of T-cell lines. Our data indicate that the % of apoptosis is significantly higher in HIV+ PBMN cells than in PBMN from healthy donors. % of apoptosis was directly correlated with CD4 cells (group A) or monocytes (group B). Furthermore, the g-GT activity of PBMN from HIV patients was significantly lower than that of PBMN from healthy donors but increased with the % of apoptosis. These results suggest that g-GT activity and apoptotic process correlate during HIV infection. Both parameters might add to the comprehension of the cellular events occurring during the progression of the disease.

S11-10

RNA-PROTEIN INTERACTIONS INVOLVED IN THE RECOGNITION OF THE PHAGE Qβ PLUS STRAND RNA TEMPLATE BY THE Qβ REPLICASE SYSTEM

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Qβ replicase holoenzyme interacts with Qβ plus strand RNA at two internal sites, the S- and the M-site. We now show by deletion analysis within the M-site region that high template activity depends on the segment nt. 2688-2787, comprising a phylogenetically conserved, branched stem-loop structure. S- and M-site interactions can be visualized by electron microscopy of chemically crosslinked complexes, which appear as looped filament structures. Deletion RNAs lacking the S-site, which are fully active templates, did not form looped structures with replicase, whereas an RNA with an M-site deletion, which is a bad template, unexpectedly formed loops involving the S-site and a site close to the deleted M-site. Looped complexes similar to those obtained with replicase were also observed with purified ribosomal protein S1 (one of three host subunits of replicase), indicating that this subunit is responsible for the binding specificity of replicase on the plus strand. Host factor, a protein essential for high template activity of plus strand RNA, forms double-looped plus strand complexes in which the 3'-end as well as the S- and the M-site (or nearby sites) are crosslinked to the protein, suggesting that binding of the 3'-end, where synthesis begins, is mediated by this protein. Deletion variant RNAs bound to host factor resulted in various looped or double-looped complexes involving the 3'-end, the M- and/or the S-site or flanking sites.

S11-11

Activation of HIV transcription by Human Foamy Virus in transgenic mice.

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We set out to investigate the possibility that the regulatory genes of HFV may act as transcriptional co-factors of HIV. To study the effects of *bel-1*, the transcriptional activator of HFV, on the HIV-1 LTR, we generated double transgenic mice for *bel-1* and for the HIV-1 LTR linked to a *lacZ* reporter gene. Moreover, to identify the *cis* acting elements mediating *bel-1* action on the HIV LTR we analyzed the consequences of deletions in the negative regulatory element (NRE) or in the NF-kB binding sites.

We demonstrate that *bel-1* is capable of activating the transcription of HIV-1 LTR *in vivo*. Such transactivational activity, however, was observed exclusively in a subset of hippocampal neurons, while cortical neurons expressing *bel-1* did not show transactivation. Transactivation was completely abolished by the deletion of the NF-kB binding sites.

Our study indicates that transcriptional transactivation of HIV-1 by HFV can be accomplished *in vivo* and is dependent on NF-kB binding sequences. It is conceivable that in a situation of co-infection this potential mechanism of cooperation between HFV and HIV attains clinical significance.

S11-12

HUMAN LEUKEMIA INHIBITORY FACTOR (LIF): PROMOTER CHARACTERIZATION AND MODULATION BY HIV-1 TAT.

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To investigate the molecular mechanisms controlling LIF gene expression, we have identified the *cis*-response elements on the promoter using transient transfections of T cell lines and CAT assays. These include a positive element, AP1 (-361/-353) and two negative regulatory elements at -541/-530 and -255/-243. In vitro studies demonstrated modulation of LIF by HIV. Cotransfection of the LIF-CAT construct with a tat-expressing plasmid resulted in a three fold induction of LIF. The tat-RE appeared to overlap with a silencer element at -542bp, since deletion or point mutations in this site abrogated the tat induced transactivation. Interestingly, this site, CAGTTCCACTT, differs only by one base from the IFNb silencer. The importance of derepression of silent cytokines in T cell lymphocytes will be discussed.

S11-13

Transport of Mutant Influenza virus Hemagglutinins During Exocytosis and Endocytosis in Polarized Epithelial MDCK cells

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The influenza virus hemagglutinin (HA) is normally targeted to the apical membrane in MDCK cells. It can be converted to a basolateral protein by changing cysteine 543 in the cytoplasmic domain to tyrosine (HAY543) , a mutation that also created an internalization signal. These two traffic signals are overlapping, but distinct. HAs in which cysteine 546 was mutated to arginine, phenylalanine, or serine were expressed in MDCK cells. Sorting of these mutants to the basolateral surface was not consistent with their endocytic rates, indicating that internalization and basolateral sorting signals are distinct. Basolateral sorting of the HA mutants was also differentially sensitive to increased protein expression in MDCK cells stimulated by sodium butyrate. Under these conditions 20-40% of HAY543 was converted to the apical surface, whereas missorting of the HAY543,R546 mutant was not affected. At the apical surface, HAY543,R546 was internalized 3-fold faster than HAY543, but was transcytosed to the basolateral surface 2-3 fold more slowly. Thus, transcytosis from the apical to the basolateral surface was not a function of the rate of entry into early endosomes. After internalization from the apical surface, HAs were sorted into transcytotic pathway to the basolateral surface with the same relative efficiency as they were sorted into the exocytic pathway to the basolateral surface. Transport of HAs to the apical surface by either the exocytic pathway from the TGN or by transcytosis from the basolateral surface was more sensitive to BFA than was transport to the basolateral surface.

S11-14

THE NEONATAL Fc RECEPTOR IS NOT REQUIRED FOR MUCOSAL INFECTION OF MOUSE MAMMARY TUMOR VIRUS

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The receptor that mediates transport of mouse mammary tumor virus (MMTV) across the intestinal barrier of newborn mice has not yet been identified. The neonatal Fc receptor (nFcR), which consists of an α chain associated with β2 microglobulin, is expressed in enterocytes during the neonatal period. Its disappearance correlates with the onset of intestinal resistance to MMTV infection. Since β2 microglobulin deficient (β2-deficient) newborn mice are unable to assemble a functional nFcR, we tested whether they were still susceptible to MMTV infection. β2-deficient pups forster nursed by MMTV infected BALB/c females,deleted the $CD4^+$ T cells population reactive to the superantigen encoded by MMTV. Since infection still occurs, this indicates that the nFcR does not mediate transepithelial transport of infectious MMTV.

S11-15

Baculovirus expression of the mouse mammary tumor virus (MMTV) envelope glycoprotein (gp52).

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Mouse mammary tumor virus (MMTV) is produced in mammary glands of infected females and transmitted to suckling new-borns through the milk. The passage of MMTV from the gut to mammary glands is poorly understood. Although the virus is able to penetrate the epithelium of the gut, viral replication seems to occur only in B lymphocytes contained in the Peyer's patches.

In order to define the nature of a potential receptor for MMTV, we expressed the envelope glycoprotein (gp52) in bacteria. Since lymphocytes seem to be the primary targets for MMTV infection and carry the virus to mammary glands, we used them in binding studies. Bacterial gp52 coupled to fluorescent latex beads shows a clear binding to B cells but not to T cells. Cross-linking experiments and affinity chromatography with this protein failed to further characterise the potential receptor for MMTV.

We have therefore decided to use recombinant baculoviruses to produce the MMTV gp52 in order to improve the affinity of the recombinant protein for its receptor. The results of this experiment will be presented.

S11-16

DEGENERATION OF THE CEREBELLAR GRANULAR CELL LAYER IN HFVDGPE TRANSGENIC MICE

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Human Foamy Virus (HFV) has been isolated from different patients with several diseases. However, it could never be identified as the causative agent of any distinct disease. HFV has the typical organization of complex retroviruses with LTR, gag, pol, env, and auxiliary genes. HFVdgpe transgenic mice carry a construct which encodes auxiliary bell and bet as well as truncated forms gag, pol, and env under transcriptional control of its own LTR. HFVdgpe transgenic mice develop a severe neurologic syndrome mainly consisting of spastic tetraparesis and blindness. These mice show a neuronal loss in CA3 of the hippocampus and in the cortex as well as a degeneration of striated muscle fibres. The severity of the phenotype in HFVdgpe transgenic mice is independent from the number of integrated copies. Besides the symptoms described above, 2 out of 8 HFVdgpe-lines developed an ataxic gait. These mice show expression of transgenic mRNA and proteins within the cerebellar granular cell layer ultimately leading to its progressive degeneration. The degeneration of granular cells is accompanied by loss of their processes. Probably due to the loss of granular cell axons, the cerebellar molecular layer is narrowed in the late stages of the disease. Even in long-time surviving mice, no anterograde or retrograde degeneration of functional dependent neuronal populations is detectable.

S11-17

Expression of PrP in PrP-less mice using an adenovirus vector

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Scrapie infectivity is associated with PrP^{Sc}, an isoform of a normal host protein, PrP^{C}. Mice devoid of PrP^{C} (*Prn-p*$^{o/o}$) develop and behave normally but are resistant to scrapie. Overexpression of *Prn-p* transgenes in *Prn-p*$^{o/o}$ mice restores susceptibility to scrapie and reduces the incubation time in a PrP dose-dependent manner. To identify regions of PrP^{C} required for scrapie agent replication and promotion of disease, mutated *Prn-p* genes were introduced into *Prn-p*$^{o/o}$ mice by transgenesis, the mice were challenged with prions and analyzed for scrapie susceptibility. This strategy requires the generation of a mouse colony for each PrP mutation to be analyzed. To circumvent this time-consuming approach, transfer of mutant *Prn-p* genes into PrP-less mice using viral vectors was undertaken as an alternative strategy. We generated adenovirus vectors (Ad) encoding PrP driven by a RSV promoter. PrP^{C} expression was elicited in tissue culture and in brain of the *Prn-p*$^{o/o}$ mouse by infection with Ad-RSV-PrP. The spread of Ad in the brain was confirmed by Ad-RSV-lacZ. To determine whether Ad-mediated PrP expression confers susceptibility to scrapie, Ad-RSV-PrP or Ad-RSV-PrP-infected neuroectodermal tissue was injected intracerebrally into *Prn-p*$^{o/o}$ mice and the mice were inoculated with scrapie. The brains are being analyzed for pathology and prion replication.

S11-18

Towards a small animal model for measles virus infection: transgenic mice expressing the viral receptor (CD46) with human-like tissue specificity

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Animal testing of measles virus (MV) vaccines and pathogenesis studies are limited to primates. MV host range restriction is primarily determined by its receptor, the cell surface molecule CD46. To obtain a mouse strain expressing CD46 with human-like tissue specificity we microinjected in oocytes a yeast artificial chromosome (YAC) with the CD46 gene at the center of a 400 kb human DNA segment (Hourcade et al., Genomics 12, 289-300). Two of 17 pups born from five animals carried the CD46 gene, and one animal transmitted it. The quantity and quality of CD46 protein isoforms detected in kidney, brain and lungs of two F_1 heterozygous mice were similar to those of the corresponding human tissues. We plan to challenge these mice with MV. We will also produce by genetic crossing a mouse line expressing CD46 but deficient in the interferon α/β receptor, and thus expected to be extremely sensitive to MV infection.

S11-19

EXPRESSION OF A SINGLE CHAIN ANTIBODY ON THE SURFACE OF VACCINIA VIRUS

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Vaccinia virus was used for centuries as a vaccine against smallpox, which finally resulted in the eradication of the disease. Novel functions might be assigned to this virus by specifically targeting it to certain cells in an organism, where it would e.g. express recombinant gene products the cell is lacking, produce specific cytokines to stimulate the immunity of the host, or simply destroy the cell by viral cytopathic action.
Therefore, as one possible model, we expressed a single chain antibody (scFv) to the tumor antigen erbB-2 on the surface of enveloped vaccinia virus particles. The viral hemagglutinin gene was modified in such a way that individual domains of the surface protein where replaced by the scFv. We will show (i) that the antibody is expressed on the surface of extracellular enveloped virus, (ii) whether it is able to bind its specific antigen, and (iii) its effect on viral infectivity to cells expressing the erbB-2 receptor. This is the first report of expression of a heterologous recognition structure on the surface of vaccinia virus.

S11-20

IDENTIFICATION OF THE VACCINIA VIRUS CELL ATTACHMENT PROTEIN

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Vaccinia virus, originally renowned as a vaccine against smallpox, has been extensively used as a vector for overexpression of heterologous proteins. In addition, vaccinia or other poxviruses have been proposed as live vaccine carriers. In these respectsd, their broad host range provides a major advantage.
On the other hand, a modified vaccinia virus exclusively infecting cells expressing a specific surface marker would provide a valuable tool to target heterologous gene expression or viral cell destruction to defined cells. Only very little is known about the first events of vaccinia infection; neither the host cell receptor nor the viral ligand for attachment have been identified so far. To envisage specific targeting of vaccinia virus, we want to determine and modify its cell attachment protein. Deglycosylation and lectin inhibition studies will show whether carbohydrates play a role in the virus-host cell interaction. Antibody inhibition experiments and biochemical binding studies using labeled viral surface proteins might then allow to identify the component responsible for the first step of infection and to define the molecular basis of host specificity.

S11-21

CRYSTALLISATION AND PRELIMINARY X-RAY STRUCTURAL DATA OF A COMPLEX OF HIV ENHANCER DNA AND ARTIFICIAL REPRESSOR

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We have designed and synthesized an HIV-1 enhancer-binding polypeptide (Städler, K. et al. (1995) Int. J. Peptide Protein Res. 46, 333-340) that contained the recognition helix of the DNA-binding domain of bacteriophage 434 repressor and that showed preferred inhibition of the in vitro transcription of HIV enhancer containing plasmids . To determine the molecular structure of the peptide-HIV enhancer DNA complex, crystallisation experiments were started. Cocrystals of the repressor peptide (1.25-1.5mM, GKTKRPRFGKTKRPRVGQQSIEQLENGKTKRPRFGKTKRPRY, recognition helix underlined) and a sticky-end 20-bp HIV enhancer DNA (1.25-1.5 mM, 5'-TGCTACAAGGGACTTTCCGC-3') were obtained in 5 mM sodium acetate, pH 4.5, NaCl, $CaCl_2$ and PEG 6000. The crystals diffracted to 5Å resolution. Preliminary data suggested that the unit cell was orthorhombic with axial dimensions about 60x70x180Å. Diffuse scattering revealed a helix with pitch about 43Å.

S11-22

RECOMBINANT BOVINE HERPESVIRUS-1 LACKING TRANSACTIVATOR PROTEIN BICP0 ENTAILS LACK OF GLYCOPROTEIN C AND SEVERELY REDUCED INFECTIVITY.

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The immediate-early transactivator protein BICP0 is a key element in regulation of the replicative cycle of bovine herpesvirus 1 (BHV-1). To create recombinant BHV-1 lacking BICP0, we cotransfected MDBK cells with BHV-1 DNA and a transfer plasmid containing a β-gal gene inserted into the BICP0 coding sequence. A recombinant (A2G2) was selected and analyzed by Southern blot and PCR to confirm that the BICP0 gene was interrupted by the β-gal gene and that wt progeny was absent. Infection with A2G2 yielded 100 to 1000 fold less infectious progeny compared to wt virus, whereas the number of viral genomes determined by quantitative PCR remained the same. Examination of A2G2-infected MDBK cells by IF and Western blots proved that BICP0 was absent. Surprisingly, glycoprotein C (gC) was also missing, but other viral proteins (gB, BICP4) were present in normal amounts. Restoration of the BICP0 gene also restored the gC^+ phenotype. Together with our earlier observations that BICP0 stimulates a gC promoter in CAT assays, the results suggest that BICP0 is required for gC synthesis. In turn, gC appears to be required for production of high-titer progeny although neither BICP0 nor gC are strictly essential for virus replication.

S11-23

A Vβ4-specific superantigen encoded by a new exogenous mouse mammary tumor virus

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The superantigen (SAg) expressed by mouse mammary tumor virus (MMTV) has been shown to play an essential role in the course of the viral life cycle. We describe a Vβ4-specific SAg encoded by a new exogenous MMTV carried by the SIM mouse strain. This is the first report of a viral or bacterial SAg reacting with Vβ4+ T cells. Injection of MMTV(SIM) in adult Balb/c mice leads to a rapid and strong stimulation of Vβ4+ CD4+ T cells. Neonatal exposure to the virus induces a progressive deletion of these same cells. In contrast to other strong MMTV SAgs, this new SAg strictly requires the presence of MHC class II I-E molecules for its presentation to T cells. Sequence analysis of the open reading frame in the 3'LTR of MMTV(SIM) revealed a new predicted amino acid sequence in the C-terminal polymorphic region. Furthermore, sequence comparisons to the most closely related SAgs with a different Vβ-specificity give hint to the specific residues important for the interaction with the T cell receptor.

S11-24

PROPERTIES OF A *CIRC* GENE DELETION MUTANT OF BOVINE HERPESVIRUS 1 *IN VIVO*

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Functionally, herpesvirus genes may be distinguished as (i) minimal essential, (ii) supplemental essential, and (iii) as genes with unknown functions. The function of the *circ* protein of bovine herpesvirus 1 (BHV-1) is unknown. *Circ* is a virion component that has been shown to be non essential for virus replication *in vitro* (Fraefel et al., J. Virol. 68, 1994).

To study the biological function(s) of *circ* in the natural host, calves were inoculated with either wild type (wt) BHV-1 or a deletion mutant containing the β-galactosidase gene instead of *circ* (rBHV-1Δ*circ*-blue).

The recombinant virus remained stable throughout the experiment. With respect to infection, virus spread within the body, and establishment and reactivation of latency rBHV-1Δ*circ*-blue behaved similar to its wt parents. Antibodies against BHV-1, however, were demonstrated earlier, and antibody titers increased faster in calves inoculated with rBHV-1Δ*circ*-blue. Interestingly, the animals inoculated with wt BHV-1 did not develop antibodies against the *circ* protein.

We conclude that *circ* is non-essential for the BHV-1 infection in its natural host. The function of *circ* may concern the development of the immune response. This hypothesis is supported by an amino acid motif found within *circ* that is present in IL-6 binding proteins of mice and rats.

S11-25

BRAIN CELL TYPE-SPECIFICITY AND GLIOSIS-INDUCED ACTIVATION OF THE HUMAN CYTOMEGALOVIRUS IMMEDIATE EARLY PROMOTER IN TRANSGENIC MICE

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Human cytomegalovirus (HCMV) can cause severe opportunistic infections in congenitally infected infants and in immunodeficient individuals. Molecular mechanisms underlying cell type-specificity of HCMV infection are poorly understood. In transgenic mouse embryos, the HCMV immediate early (IE) promoter directs *lacZ* gene expression specifically to sites corresponding to target tissues of congenitally infected human fetuses. Thus, factors regulating the IE promoter appear to be conserved across species and may be important for determining HCMV infection targets. Here, we have analyzed cell type-specific HCMV-IE promoter activity in the brain of transgenic mice to identify potential HCMV infection targets in the CNS. Immunohistochemical analysis revealed IE-lacZ expression in choroid plexus, endothelial and ependymal cells, and neurons in several discrete regions. The IE promoter was inactive in astrocytes, but was dramatically induced in reactive astrocytes following a stab lesion of the neocortex. These results are consistent with clinical data on human HCMV brain infection and suggest a model for HCMV progression in the CNS, whereby astrocytes become permissive for infection in response to tissue damage caused by infection of primary targets.

S11-26

Use of RT-PCR as a Rapid Screening Method for pathogenic Viruses in Water

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We developed a rapid, efficient and inexpensive method to concentrate enteroviruses, rotaviruses and hepatitis A viruses from artificially spiked tap and surface water samples. Following specific conditioning of water, viruses were efficiently bound to SiO_2. After concentration of virus-SiO_2-complexes through centrifugation, viral RNA was extracted with guanidine thiocyanate. Environmental inhibitors of reverse transcriptase and Taq polymerase were removed with several washing steps. Subsequent RT-PCR allowed the detection of as little as 1 tissue culture infective dose of virus in 1 L water samples within 36 h. Field studies with this method revealed, that several rivers, lakes, wells and fountains in north-east Switzerland are contaminated with viruses. Some water samples polluted with viruses, were free of indicator bacteria for fecal contamination.

S11-27

ANALYSIS OF MEASLES VIRUSES CARRYING MUTATIONS IN THE EDITING REGION

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The Measles Virus (MV) P gene is transcribed into two mRNAs encoding the P or the V protein. The V mRNA is produced by insertion of a single G residue probably triggered by stuttering of the viral polymerase complex at the 3'UUUUUCCC5' editing region. To analyse the editing mechanism and the function of the V protein, mutations have been introduced into the cDNA giving rise to full-length antigenomic MV RNA. Recombinant MVs could be generated by two different rescue systems. Two MVs with U to C transitions at different positions do not produce V protein, due to their inability to edit the P mRNA. However, their plaque sizes, growth rates and final titres do not differ from the MV Edmonston B strain. Another virus containing 6 rather than 3 C residues produces elevated levels of V protein. Syncytia generated by this virus lyse 1-2 days later than usually and higher final titres are reached. This suggests a regulatory function of the V protein in viral RNA replication.

S11-28

FUNCTIONAL ANALYSIS OF MEASLES VIRUS ENVELOPE ASSEMBLY

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The viral envelope proteins F and H are preferentially incorporated into measles virus (MV) particles. It is likely that F and/or H protein interactions with the cytoplasmic membrane-associated M protein induce clustering of the envelope proteins into areas of virus assembly. Analysis of protein-membrane interactions indicated that besides the integral transmembrane proteins F and H, a significant fraction of the M protein (≈25%) as well as of the RNA genome encapsidating N and P proteins (≈15%) associate with intracellular membranes. The M protein-membrane complex was shown to be stable and to form independently from the coexpression of other viral proteins. To determine regions within the cytoplasmic domains of the envelope proteins interacting with the M protein we used homologue-exchange and deletion mutagenesis. We showed that with one exception deletion or replacement of the cytoplasmic domains neither affected transport of mutant proteins nor fusion function. By a reverse genetics approach (Radecke et al., EMBO J. 14, in press), the above mutants are now being tested for their competence to form infectious viral particles.

S11-29

THE ROLE OF THE C-TERMINAL REGION OF Mx PROTEINS

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Mx proteins are GTPases with intrinsic antiviral activity. They form large oligomers but the role of these structures in antiviral activity remains elusive. We show here that an amphipatic helix (LZ2) located in the C-terminal region of Mx exerts a dominant negative effect on wildtype Mx. Experiments employing the two-hybrid system show, that LZ2 interacts strongly with the N-terminal region of Mx. Co-expression studies revealed that this association is based on intermolecular interactions, demonstrating that LZ2 is necessary for the oligomerization of Mx. Mutations abrogating the amphipatic character of the helix not only destroyed the dominant negative effect of the C-terminus and the interaction with the N-terminal region, but also abolished the GTPase activity of Mx. This indicates that the formation of inter and/or intramolecular head-to-tail structures mediated by LZ2 is required for the GTPase activity and hence the antiviral function of Mx.

S11-30

A STABLY TRANSFORMED HeLa CELL LINE WHICH EXPRESSES FUNCTIONAL BICP0 UNDER TETRACYCLINE CONTROL IS ABLE TO TRANSACTIVATE LTR OF HIV-1

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We have established a cell line in which the expression of bICP0, a principal transactivator of gene expression of Bovine herpesvirus 1 (BHV-1), can be regulated both positively and negatively. For this, a hybrid promoter containing the tet-operon (tet-O) and a minimal αTIF responsive element was fused to the body of the bICP0 gene and used to transform a HeLa cell line (Gossen and Bujard, PNAS, 1992) which already expressed a fusion protein consisting of the repressor (tet-R) of the tet-O and the carboxyterminus of αTIF, a potent transactivator.

PCR analysis confirmed the establishment of such cell lines with bICP0 gene(s) in their genomes and immunofluorescence indicated that bICP0 was synthesized after tetracycline removal. The functional activity of bICP0 in these cells was demonstrated by CAT assays. The induced cell lines transactivated the native bICP0 promoter as well as the herpes simplex virus thymidine kinase promoter. In contrast, the promoter for bICP22 of BHV-1 was repressed under the same conditions. Even in the absence of *tat*, the major transactivator of retroviral genomes, CAT genes fused to the long terminal repeats (LTR) of human immunodeficiency virus (HIV) were transactivated in the induced cells in a dose dependent manner. This may be of interest regarding research on the pathogenesis of diseases which are linked to both herpes- and retrovirus infections.

S11-31

BIOCHEMICAL AND BIOLOGICAL PROPERTIES OF THE AAV REP PROTEINS: A HIGH MOBILITY GROUP CHROMOSOMAL PROTEIN IS AN ACCESSORY FOR AAV REP DNA BINDING.

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Adeno-associated virus (AAV), and in particular the virus-coded regulatory protein Rep, has been shown to have an oncosuppressive effect. Analysis of the binding of Rep to a DNA sequence in the AAV terminal repeat by electrophoretic mobility shift assay indicated that DNA protein complexes were formed. The initial experiments also suggested that Rep binding to DNA is enhanced by an accessory factor. We identified the factor as a member of the high-mobility-group chromatin proteins. To test whether HMG proteins affect Rep function, we examined site-specific DNA cleavage, one of the reactions Rep is believed to catalyse during AAV DNA replication. Cleavage *in vitro* was greatly stimulated by purified HMG-1/2. Therefore the HMG proteins can stimulate both the binding and the cleavage of AAV DNA by Rep.

S11-32

ARTIFICIAL VIRUS-LIKE PARTICLES FORMED FROM THE PAPILLOMAVIRUS HPV-18 CAPSID PROTEINS.

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Human papilloma viruses (HPV) are closely associated with certain cancers, notably cervical carcinoma. It is difficult to obtain viral particles for study because these viruses do not grow in cultured cells. To overcome this problem, we synthesised the capsid proteins of HPV-18, L1 and L2, using recombinant vaccinia virus- and baculo virus-based expression systems. Virus-like particles could be isolated from infected cells and purified by sucrose and CsCl gradient centrifugation. Well-defined assemblies of capsomers into virus-like structures with a diameter of about 55 nm were observed by electron microscopy. When particles containing L1 and L2 were assembled in cells containing episomal DNA, DNA was found associated with the purified particles. We are now testing whether the DNA is encapsidated and what the requirements are for this to occur.

S11-33

Nature of mouse hepatitis virus RNA recombination between defective interfering particle and helper virus

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We attempted to introduce site-specific mutations into the spike gene of MHV by using homologous recombination between synthetic defective interfering (DI) RNA and helper virus RNA. The enterotropic strain MHV-RI spike gene cDNA was cloned into pMIDI, a full length cDNA clone of respiratory strain MHV-A59, to yield pDPRIS. MHV-A59 and the pDPRIS were co-passaged several times in cell culture. Using a highly specific and sensitive RT-PCR, a recombinant-specific 1.65 kb signal was detected in intracellular and viral RNA. Three PCR products of correct size were cloned and sequenced. The results showed that there are different species of recombinant RNAs which indicates that crossover sites are randomly distributed over the examined 1.65 kb region of the S gene. We suggest that recombination happened via a crossover from genomic RNA template to the synthetic DI RNA template and back again at different locations, generating a series of recombinant RNAs. This result confirmed the previous observations that MHV RNA recombination is common and random in nature. The PCR results may indicate the presence of recombinant viruses with chimearic spike proteins, a matter which we are currently trying to confirm.

S11-34

TICKS ZOONOSES IN THE SOUTHERN PART OF SWITZERLAND (TICINO): FAUNISTICAL AND EPIDEMIOLOGICAL ASPECTS.

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The diversity and the distribution of tick species and their infection rate by the pathogenic microorganisms *Borrelia burgdorferi* sensu lato (etiologic agent of Lyme disease) and *Rickettsia sp.*(agent of spotted fever), were studied in Ticino. PCR analysis was performed for the detection of *Borrelia* spirochetes in *Ixodes ricinus* and *Ixodes hexagonus*, and the hemolymph test was done on *Rhipicephalus sanguineus* for the detection of *Rickettsia sp.* PCR analysis revealed a moderate rate of infection (around 2%) with *B. burgdorferi* sensu lato in both vectors *I. ricinus* and *I. hexagonus*. These results are in agreement with the modest number of Lyme borreliosis cases yearly recorded in Ticino. Further, through analysis of DNA sequences, the strains carried by the infected ticks were identified as belonging to the genomic group VS116. The wide finding of the Mediterranean species *R. sanguineus* in different locations confirms its establishement in Ticino. One specimen was even infected with *Rickettsia sp.*

S11-35

MOLECULAR PHYLOGENY OF THE AGENTS OF LYME DISEASE *BORRELIA BURGDORFERI* SENSU LATO.

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Several aspects of the phylogeny, taxonomy, and clinical relevancy of the spirochetes *Borrelia burgdorferi* sensu lato, the causative agents of Lyme disease, have been addressed. The genetic structure of *Borrelia* populations has been elucidated by analyzing molecular data produced with multilocus enzyme electrophoresis and DNA sequence analysis of the *hbb* gene, encoding a member of the histone-like proteins. Computational analysis of both types of data lead to similar phylogenetic trees, revealing a complex genetic structure of the *B. burgdorferi* sensu lato population. According to our genetic analysis this population is composed of three pathogenic species (*B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii*) two newly proposed species (*B. japonica* and *B. andersonii*) and three new genomic species (corresponding to groups DN127, PotiB2 and VS116). Further, the different chronic manifestations of the disease could be associated to the three pathogenic species.

S11-36

***BORRELIA BURGDORFERI* AND THE ETIOLOGY OF ALZHEIMER DISEASE: A CONTROVERSY.**

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The involvement of spirochetes, such as the etiologic agent of Lyme disease, *Borrelia burgdorferi*, in Alzheimer's disease (AD), a common neuropathology, has been proposed by several groups in the past. In our laboratory, brain autopsies from 20 AD patients were analyzed for the presence of *B. burgdorferi* sensu lato by both standard and nested PCR techniques based on various target regions. In addition, Western blot and ELISA tests for the detection of antibodies against spirochetal antigens were performed on 27 clinical AD patients' sera. By these methods, we did not obtain any evidence of the involvement of *B. burgdorferi* in this neuropathology.

S11-37

INBRED STRAIN DERIVED MURINE EMBRYONIC STEM CELL LINES

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Most of the germ-line competent ES cell lines used so far in gene targeting experiments have been derived from blastocysts of sublines of the mouse strain 129/Sv. Recently, ES cell lines that have been derived from the C57BL/6 inbred mouse strain were successfully used to generate knock-out mice. In addition, we have isolated several male ES cell lines from each the BALB/c, DBA/2 and C3H inbred strains. One of the BALB/c ES cell lines has been used to generate IL-4 deficient BALB/c mice. Of 4 male DBA/2 ES cell lines, two were transmitted through the germ-line and one is currently being used in gene targeting experiments. The C3H ES cell lines are currently being analyzed. With the availability of these ES cell lines it is now possible to induce a mutation on the genetic inbred background of choice and to analyze the induced mutation in different genetic backgrounds without laborious breeding.

Synaptic Release

S12-01

MOLECULAR AND FUNCTIONAL DIVERSITY OF INDIVIDUAL NERVE TERMINALS OF ISOLATED CORTICAL NEURONS.

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Learning is associated with changes in the strength of connections between neurons which can occur independently in individual synapses of the same cell. A possible presynaptic component of these adaptive changes is suggested by the differential expression, in vivo, of nerve terminal proteins (NTPs) involved at various stages of membrane fusion and transmitter exocytosis. Here we show that single cortical neurons cultured in isolation are capable of differential expression of NTPs at different synapses. Using the dye FM1-43 in mixed neuronal cultures, we also show that the intensity of uptake and release of the dye following K^+ stimulation at each synapse correlate with the levels of synaptophysin, synapsin I and SV2 but not of synapsin II. These data demostrate that differential expression of NTPs is predictive of synaptic strength. They also show that physiological variations of the levels of expression of synaptophysin, synapsin I and SV2 correlate with synaptic efficacy.

S12-02

SNARE Protein-Protein Interactions in Adrenal Chromaffin Cells

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Specificity of interaction between secretory vesicles and the plasma membrane in neurotransmission is thought to be determined by the interaction of the vesicle- and target membrane-associated SNARE proteins synaptobrevin, and SNAP-25 and syntaxin, respectively. *In vitro*, recombinant SNARE proteins form stable trimeric complexes that dissociate upon addition of SNAPs and NSF in the presence of MgATP.

We have investigated the role of t-SNARE heterodimer formation *in vivo* by using permeabilized chromaffin cells as a model system. We could show that syntaxin 1A, and peptides containing the full carboxyterminal heptad repeat region, bind with high affinity to endogenous SNAP-25. Synaptobrevin of the chromaffin granules does not bind to endogenous syntaxin or SNAP-25, but to complexes of syntaxin and SNAP-25. In the plasma membrane of chromaffin cells, only a small proportion of SNAP-25 is found in a complex with syntaxin, probably because the majority of syntaxin is associated with mSec1. We suggest that docking of chromaffin granules induces dissociation of mSec1 from syntaxin, to allow its association with SNAP-25. This t-SNARE heterodimer formation can be blocked by heptad repeat-containing peptides of syntaxin, that themselves are able to form a complex with plasma membrane SNAP-25 and synaptobrevin of the chromaffin granules. These peptides, by mimicking plasma membrane-anchored syntaxin, inhibit secretion of noradrenaline in permeabilized chromaffin cells. We thus conclude that dimerization of the two endogenous t-SNAREs is an essential step preceding docking and/or fusion of chromaffin granules.

S12-03

The SNARE complex and synaptic vesicle docking: an open ended question.

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Neurotransmitter release occurs via Ca^{2+} triggered fusion of synaptic vesicles (SV) with the presynaptic plasmamembrane (PM). Vesicles that appear stably docked at the presynaptic active zone are thought to constitute the readily releasable pool that are activated by calcium. *In vitro*, the SV protein synaptobrevin associates with the PM proteins syntaxin and synaptosomal associated protein of 25 kDa (SNAP-25). In turn these three proteins (SNAREs) act as receptors for the soluble NSF attachment protein (SNAP) that allows the subsequent binding of the ATPase N-ethylmaleimide sensitive fusion protein (NSF). NSF hydrolyses ATP to disrupt the macromolecular complex. The association of the SNAREs and their subsequent activation by NSF has been postulated to function in both the docking and fusion of synaptic vesicles (Söllner et al. 1993, Cell 75. 409). This hypothesis appears at odds with observations that tetanus toxin, which inhibits neurotransmitter release by cleaving synaptobrevin, does not decrease the number of docked vesicles in treated terminals (Hunt et al., 1994 Neuron 12. 1269). We have investigated this conflict by analysing the effect of clostridial neurotoxins on distinct intermediates of synaptic vesicle fusion that can be isolated in vitro. We have shown SNAPs and NSF bind to, and upon ATP hydrolysis, disrupt an SDS resistant complex which forms when the three SNAREs associate with cachother (Hayashi et al., 1994 EMBO J 13 5051). Our observations highlight the physiological relevance of this intermediate. Furthermore, tetanus toxin treatment compromises the stability of the SDS resistant complex without perturbing its interactions with SNAP and NSF, suggesting the intrinsic stability of this intermediate is essential in producing fully functional NSF activated intermediates. Interestingly both fragments of tetanus toxin cleaved synaptobrevin are found in these destabilized complexes leaving open the possibility that the cleaved protein could participate in a SNARE dependent mechanism of synaptic vesicle docking.

S12-04

ACTIONS OF CLOSTRIDIAL NEUROTOXINS ON SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS

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The functional role of SNARE proteins in the mammalian CNS has been investigated by means of clostridial neurotoxins. Pre-treatment of rat hippocampal slice cultures with botulinum neurotoxin (BoNT) type F (40ng/ml for 48-72 h), which cleaves both isoforms of the SNARE protein synaptobrevin, strongly reduced (on average by 10 times) the frequency, but not the amplitude, of miniature excitatory postsynaptic currents (mEPSCs) as well as the amplitude of EPSCs evoked with stimulation in the dentate gyrus, recorded from whole-cell voltage-clamped CA3 pyramidal cells. Application of three secretogogues, α-latrotoxin (ltx), the Ca^{2+} ionophore ionomycin, or the protein kinase C (PKC) stimulator phorbol ester to BoNT/F-treated cultures failed to produce the increase in mEPSC frequency observed in control cultures. We conclude that synaptobrevin plays an essential role in: 1) the spontaneous fusion of synaptic vesicles with the presynaptic membrane, 2) fusion in response to Ca^{2+} influx mediated by either action potentials or ionomycin, 3) fusion that has been rendered independent of Ca^{2+} influx by either PKC activation or ltx application.