also demonstrated that upon superinfection, the viral RNA genome of the viral particles was not transferred into the cytoplasm of BVDV-infected cells. Using newly developed system involving rapid generation of the MDBK cells expressing BVD viral proteins, we subsequently found that expression of the viral structural proteins was dispensable for a block occurring at the level of viral RNA replication, but required for a exclusion at the level of viral entry step. Furthermore, our data also showed that superinfection exclusion did not take place when BVDV-infected cells were passaged and become persistent. The failure of BVDV superinfection exclusion resulted from cellular alteration/adaptation of the persistently infected MDBK cells, but not from mutations in the BVD viral genome during passages. Altogether, these findings provide evidence that the superinfection exclusion of BVDV occurs not only at the level of viral replication in which the viral replicase are involved, but also at the level of viral entry with which the viral structural proteins are associated, and that a cellular factor(s) play an essential role in this process.

SL309

HIV-1 Vaccine Development: Need for New Directions.

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The AIDS epidemic continues unabated in many part of the world. After near two decades, no vaccine is available to combat the spread of this deadly disease. Much of the HIV-1 vaccine effort during the past decade has focused on the viral envelope glycoprotein, largely because it is the only protein that can elicit neutralizing antibodies(Nabs). Eliciting broadly cross-reactive Nabs has been a primary goal. The intrinsic genetic diversity of the viral envelope, however, has been one of the major impediments in vaccine development. We have recently completed a comprehensive study examining whether it is possible to elicit broadly acting Nabs by immunizing monkeys with mixtures of envelope proteins from multiple HIV-1 isolates. We compared the humoral immune responses elicited by vaccination with either single or multiple envelope proteins and evaluated the importance of humoral and non-humoral immune response in protection against a challenge virus with a homologous or heterologous envelope protein. Our results show that (1) Nab is the correlate of sterilizing immunity, (2) Nabs against primary HIV-1 isolates can be elicited by the live vector-prime/protein boost approach, and (3) polyvalent envelope vaccines elicit broader Nab response than monovalent vaccines. Nonetheless, our findings clearly indicate that the increased breadth of Nab response is by and large limited to strains included in the vaccine mixture and does not extend to heterologous non-vaccine strains. Our study strongly demonstrates how difficult it may be to elicit broadly reactive Nabs using envelope proteins and sadly predicts a similar fate for many of the vaccine candidates currently being evaluated in clinical trials. We have started to evaluate other vaccine candidates (*e.g.* genetically modified envelope proteins) that might elicit broadly reactive Nabs. We are also exploring other vaccine strategies to elicit potent cytotoxic T lymphocyte responses. Preliminary results from some of these experiments will be discussed.

SL310

Nrg1 is a Transcriptional Repressor for the Expression of Genes Involved in Glucose Metabolism in

Saccharomyces cerevisiae

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Expression of genes encoding starch-degrading enzymes is regulated by glucose repression in the yeast *Saccharomyces cerevisiae.* We have identified a transcriptional repressor, Nrgl, in a genetic screen designed to reveal negative factors involved in the expression of *STA1,* which encodes a glucoamylase. The *NRG1* gene encoeds a 25-kDa C2H2 zinc finger protein which specifically binds to two regions in the upstream activation sequence of the STA1 gene, as judged by gel retardation and DNase I footprinting analyses. Disruption of the *NRG1* gene causes a fivefold increase in the level of the *STA1* transcript in the presence of glucose. The expression of *NRG1* itself is inhibited in the absence of glucose. DNA-bound LexA-Nrgl represses transcription of a target gene 10.7-fold in a glucose-dependent manner, and this repression is ablished in both *ssn6* and *tupl* mutants. Two-hybrid and glutathione S-transferase pull-down experiments show an interaction of Nrgl with Ssn6 both in vivo and in vitro. These findings indicate that Nrgl acts as a DNA-binding repressor and mediates glucose repression of the STA1 gene expression by recruiting the Ssn6-Tupl complex. Furthermore, in northern blot analysis, the mRNA level of *SUC2, PCK1, HXT2* in *nrgl* null mutant was relieved from glucose repression in repressed condition. Therefore it is thought that *NRG1* may be concerned in glucose repression of these genes.

SL311

Development of Non-protoplast Transformation System in *Aspergillus oryzae*

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Aspergillus oryzae is a filamentous fungus classified in the group *Aspergillaceae Ascomycetes.* It is an important microorganism for industrial production of enzymes and fermented food productions. It secrets large quantities of proteins or enzymes into the culture medium which makes this organism appealing for the production of heterologous proteins. Recently electric field-mediated transformation method, electroporation, has been applied to fungal transformation. In this study, fungal transformation was carried out by bypassing the protoplast isolation step, decreasing the culturing time and non-protoplast transformation for the increment of transformation efficiency. Transformants were obtained with electroporation in optimal condition 2,500 voltage, 1,540 ohm and 0.50 capacitance. More than 1,000 transformants were obtained with 6-10 hrs cultured mycelia without enzyme treatment, called non-protoplast transformation.

SL312

The Biofuel Cell: Development of New Materials for Composing Electron Mediator-free and Electrochemical Active Acteria-free Biofuel Cell

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In this study biofuel cell is classified