

F045

Functional Analysis of Flo8 Protein and Activation of *FLO11* and *FLO1* Expression by Flo8-Mss11 Heterodimer.

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Flo8 and Mss11 play important roles in the expression of *STAI*, *FLO11*, and *FLO1* which encode an extracellular glucosylase and two cell surface proteins. The *flo8Δ* and *mss11Δ* mutants show reduced *lacZ* expressions from the *FLO11* and *FLO1* promoters and have defects in haploid invasion and flocculation. Here we show that Flo8 has the two distinct domains, the LisH motif and the activation domain on its extreme N- and C-terminus, respectively. Furthermore, we also reveal that the N-terminal region of Flo8, LisH motif is required for interaction with Mss11. GST pull-down experiments show that they can directly form a heterodimer or a homodimer which is capable of binding to DNA. Finally, we also found that Snf6, a component of the Swi/Snf complex interacts functionally and physically with both Flo8 and Mss11 and is required for Flo8- and Mss11-dependent activation.

F047

Isolation of Novel mRNA Export Factors, Rsm1 and spNic96, in *Schizosaccharomyces pombe*

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In eukaryotes, transport of mRNA out of the nucleus occurs through the nuclear pore complex (NPC) embedded in the nuclear envelope, and is mediated by soluble transport receptors. The best candidate for an mRNA export receptor is a heterodimer, NXF-NXT. We have used synthetic lethal genetic screen in *Schizosaccharomyces pombe*, in order to identify mutations in genes that are functionally linked to *mex67* (yeast homolog of NXF). Three mutations that are synthetic lethal in combination with the *mex67* null allele were isolated and define in separate complementation groups. These mutants exhibited the accumulation of poly(A)⁺ RNA in the nucleus in restriction condition, suggesting that the mutations cause the defect of mRNA export out of the nucleus. We isolated two genes by complementation of synthetic lethal mutants. The one gene (we named it as *rsm1*) encodes a predicted intronless 297-amino acid ORF of which function is unknown. And the other gene encodes a homologue of *S. cerevisiae* nucleoporin, Nic96.

F046

Dual Mechanisms of Pestiviral Superinfection Exclusion at Entry and RNA Replication

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For many viruses, primary infection has been shown to prevent superinfection by a homologous second virus. In this study, we investigated superinfection exclusion of bovine viral diarrhoea virus (BVDV), a positive-sense RNA pestivirus. Cells acutely infected with BVDV were protected from superinfection by homologous BVDV but not with heterologous vesicular stomatitis virus. Superinfection exclusion was established within 30 to 60 min but was lost upon passaging of persistently infected cells. Superinfecting BVDV failed to deliver a translatable genome into acutely infected cells, indicating a block in viral entry. Deletion of structural protein E2 from primary infecting BVDV abolished this exclusion. Bypassing the entry block by RNA transfection revealed a second block at the level of replication but not translation. This exclusion did not require structural protein expression and was inversely correlated with the level of primary BVDV RNA replication. These findings suggest dual mechanisms of pestivirus superinfection exclusion, one at the level of viral entry that requires viral glycoprotein E2 and a second at the level of viral RNA replication.

F048

Expression and Purification of the Capsid Protein of the Japanese Encephalitis Virus and Production of Its Polyclonal Antibody

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Japanese encephalitis virus (JEV) is a member of *Flaviviruses*, transmitted by mosquitoes. The core of JEV is composed of the capsid (C) proteins. In order to produce the recombinant viral C protein and the antiserum specifically recognizing the JEV C protein in this study, we have expressed and purified the JEV C protein as a Glutathion-S-Transferase (GST) fusion protein in *E. coli*. The JEV C protein-coding region has been PCR amplified using the infectious cDNA of a JEV Korean isolate, and the amplicons have been cloned into the pGEX4T-1 *E. coli* expression vector. GST-C fusion proteins were purified using a glutathione sepharose column. Subsequently, the GST-C fusion proteins were used for immunization with rabbits, and the antisera were obtained from these immunized animals. Western blot analysis using the JEV-infected BHK21 cell lysates showed that these antisera specifically reacted with the JEV C proteins. Thus, this study will provide a useful reagent for the diagnosis and understanding of the viral morphogenesis in the JEV-infected cells.