CO-EXPRESSION OF cFOS AND cJUN PROTEINS USING THE BACULOVIRUS EXPRESSION SYSTEM

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The products of the proto-oncogenes *c-fos* and *c-jun* play an important role in cell growth control, differentiation and malignant transformation. The nuclear phosphoproteins cFos and cJun associate to form the AP-1 transcriptional complex that selectively binds to gene promoters containing the AP-1 consensus sequence. Purified oncogenic proteins are essential tools in cell growth control studies. *Autographa californica* nuclear polyhedrosis virus (AcNPV) is the prototype of a helper-independent baculovirus expression vector developped by Summers and co-workers (Smith , GE, Fraser, MJ & Summers, MD. 1983. *J.Virol., 43:* 584-593) for high-level expression of recombinant proteins in *S. frugiperda* insect cells. Here we describe the expression and characterization of a mouse cFos recombinant protein using the baculovirus system as well as the ability to co-express cFos and cJun proteins in insect cells. Sf9 cell cultures were infected with a recombinant baculovirus containing the *c-fos* gene (VL1392fos) at a high multiplicity of infection (m.o.i.=10). The non-fusion cFos protein produced was compared to a hexahistidine fusion cFos (His-cFos) recombinant protein. Both proteins display similar properties as those of native mammalian cFos , namely, phosphorylation, ability to complex with Jun proteins and binding of the heterodimer to the AP-1 element. A recombinant baculovirus containing the mouse *c-jun* gene fused to a hexahistidine peptide (VLMH6jun) together with VL1392fos were used to co-infect Sf9 cells. Gel retardation assays show that insect cells are able to co-express cFos and cJun proteins forming a functional active AP-1 complex. The possibility to recover biologically active AP-1 complex using the baculovirus system greatly facilitates purification of these proteins and their use in structure-fuction relashionship studies and functional assays.

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QUANTITATION OF HPV GENOMES IN BIOLOGICAL SPECIMENS BY USI LS-PCR

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Infection with certain Human Papillomaviruses (HPV) types plays a role in development of cervical cancer. It has been suggested that the number of viral geno present in infected cells may correlate with the severity and progression of disease. Sinc simple method for the precise determination of viral copy number is not available, propose the use of Low-Stringency PCR for such quantification (Caballero et al, Nuc Acids Research, in press). The reaction involves the use of general primers GP5 and C (van den Brule et al, Int. J. Cancer 45:644, 1990), that amplify a DNA fragment aproximately 140 bp from the L1 gene of a broad spectrum of HPV genotypes. Under l stringency conditions, a HPV-specific band and several bands (LSPs) from Low-stringe annealing events with human DNA are produced. The ratio of the intensity between HPV-specific band and one of the LSPs was calculated, and standard curves generated amplifying mixtures containing different amounts of a plasmid harbouring the entire HF 16 genome and human DNA. The HPV copy number of biological specimens (cervi carcinomas and cytomorphologically normal cervical scrapes) previously known to cont HPV DNA could be extrapolated from these curves. The quantitation of HPV genomes v certainly improve our understaning of the pathogenesis of the HPV- associated lesions.

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THE MOLECULAR BIOLOGY OF GEMINIVIRUS

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The Geminiviruses are characterized by their unique twinned icosahedral particle morphology and one or two circular single-stranded DNA molecules which replicate via double-stranded intermediates in the nuclei of infected cells. Besides being agronomically important plant pathogens, this large group of plant viruses have been of interest as potential for construction of plant vectors. Members of this group can be placed into three subgroups on the bases of genome structure, vector type, and host range. Subgroup 1 (e.g. MSV - Maize streak virus, WDV - wheat dwarf virus) are transmitted by leafhoppers, infect monocotyledonous plant species, and have monopartite genomes. Subgroup II has a sole member, beet curly top virus (BCTV), which also has a genome of a single component, is leafhopper transmitted, but infects dicotyledonous plant host. Members of subgroup III are transmitted by whiteflies, has two DNA components, and infect dicotyledonous plant hosts. Some members of this subgroup are bean golden mosaic virus (BGMV), tomato golden mosaic geminivirus (TGMV), African cassava mosaic virus (ACMV), and squash leaf curl virus (SqLCV). Cloning and sequence analysis of ACMV and TGMV clearly established the bipartite nature of their genomes (designated A and B) and their size of approximately 2.6 kb each. When the other isolates were analyzed, the genomic organization was very consistent. The surprising side came from the analysis of leafhopper transmitted MSV, which had only one component, of approximately 3,7 kb, which carries all the necessary and sufficient genes for infectivity. This genome organization has also been verified for other leafhopper transmitted geminiviruses. Aspects related to the phylogeny and the diversity of the group will be addressed. Recent progress made on the mechanisms of viral replication and systemic movement as well contribution of these findings to disease symptoms and disease development will be discussed.

DEFECTIVE INTERFERING GENOMES ASSOCIATED WITH PLANT VIRUSES

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Among the defective virus genomes, the most biologically important on are defective interfering particles (DI particles). They consist of subgenon deletion mutants which have lost essential segments of the viral genome. T viral genome deleted may vary from a small number of nucleotides to over 90% the genome. The DI genome are "helver-dependent", that monno that they v replicate in the cells when co-infected with the wild type virus. The latter v deliver the deleted genetic functions of the DI-genomes. In addition, the genomes suppress the replication of wild type virus by diverging virus-suppli gene products toward DI replication and away from wild type virus replicatic Therefore, DI particles interfere with the replication of the wild type virus and a often associated to disease modulation in susceptible hosts.

For plant viruses, phenomena as symptom attenuation and virus interferen have been documented much less extensively than for animal systems. A fe reports describe the occurrence of defective viruses or genuine DI RN/ associated with plant virus infection. They include viruses found in plants infect with (negative-strand) rhabdoviruses and tospoviruses. DI RNA segments ha also been reported for wound tumor virus, and for two related groups, tl tombusviruses and carmoviruses which have positive-stranded RNA genome Within these virus groups DI RNAs have been reported for tomato bushy slu virus (TDGV), TC virus
and cymbidium ringspot virus (CyRSV). The possible role of DI RNAs durir natural infection of plant viruses and the use of these defective molecules obtain virus protection will be discussed.

MOLECULAR BASIS OF PLANT VIRUS CLASSIFICATION

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Plant viruses have traditionally been classified into different taxonomic groups (taxa) on the basis of particle morphology, serology, genome type, and biological properties such as host range, vector type and disease symptoms. During the past ten years for most plant viral taxa a growing number of representative has been studied in detail in terms of complete nucleotide sequences of their genome and high resolution crystallographic analyses of their capsids. With this flood of molecular data, it has become possible to compare groups of viruses on the basis of nucleotide and protein sequences. Such comparisons have led to the identification of unespected genetic relationships between groups of viruses which are ecologically separated by host range. Thus in plants RNA viruses are found which are more closely related to the picornaviruses and the alphaviruses of animals than to other plant viruses. This has led to the concept of "superfamilles" in which such on molecular level related, but in biological aspects very distinct viruses are harboured. Complications in setting up a phylogenetic virus taxonomy, as well as possible evolutionary mechanisms which may underly the surprising genetic relationships among modern viruses, will be discussed.

DESIGN AND ANTIVIRAL PROPERTIES OF INFLUENZA VIRUS NEURAMINIDASE INHIBITORS

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During the infection cycle of influenza virus, progeny virions bud from the plasma membr of infected cells. At that point they are potentially immobilised by virtue of interactions betw the viral haemagglutinin and sialic acid which is found in glycoconjugates on the cell surf and on glycoproteins of the virus itself. Neuraminidase inhibitors might therefore be expec to have an antiviral action by slowing release and subsequently reducing the viral burden infected hosts.

The three-dimensional structure of the influenza virus neuraminidase has been determi by X-ray crystallography to a resolution around 2Å. The structure demonstrates that str variation has not yet been observed to include active site residues. Studies of the structure complexes between the substrate and substrate analogues with the enzyme reveal the meth of enzyme-substrate interaction. The structure of the enzyme active site has been used to dir the design of new inhibitors of the enzyme. These inhibitors block multi-cycle replication virus in tissue culture and have antiviral properties in animal models.

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