VIRUSES OF EUKARYOTIC GREEN ALGAE

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PROGRESS REPORT

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SUMMARY STATEMENT. We have isolated and partially characterized many large polyhedral, dsDNA containing (ca. 330 kb), plaque forming viruses which infect a unicellular, eukaryotic, chlorella-like green alga. The plaque assay, the ability to synchronously infect the host, the short life cycle, and the ability of the viruses to undergo homologous recombination make them excellent model systems for studying many plant cell functions in the manner that bacterial and animal viruses have been used to study bacterial and animal cell functions. These viruses have several unique features including: (i) coding for DNA methyltransferase and site-specific (restriction) endonucleases and (ii) unlike other viruses, these viruses appear to code for the enzymes involved in the glycosylation of their glycoproteins.

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1. Structure of the virus genome.

The prototype chlorella virus PBCV-1 contains a large (330 kbp) dsDNA genome with a 40% G+C content. Initial studies indicated that the PBCV-1 genome might be a linear circularly permuted molecule. However, studies in the past two years have established that the genome is a linear nonpermuted molecule and that the termini (telomers) contain identical inverted repeats. Furthermore, the termini have covalently closed hairpin ends.

The termini (minus the hairpin) were cloned and sequenced to within about 40 to 60 bases of the hairpin end (appendix 2). The termini contain identical inverted repeats of at least 2,185 bases,

after which the sequence diverges. The inverted repeats contain two small open reading frames (ORFs) and several direct repeats. However, neither of the ORFs nor the remainder of the inverted repeats appear to be transcribed during PBCV-1 replication. The remainder of the PBCV-1 genome contains primarily unique DNA and has the potential to code for several hundred proteins.

Thus the chlorella viruses are similar to Frog virus 3 (an iridovirus) and African swine fever virus (recently removed from the iridovirus family) in morphology and similar to African swine fever virus and vaccinia virus (a poxvirus) in genome structure. The chlorella viruses have recently been given family status with the name Phycodnaviridae.

2. Virus encoded DNA modification and restriction endonucleases.

Several of the chlorella virus encoded restriction and modification enzymes have been isolated and characterized over the past 3-4 years (see Table 2 in appendix 3). Furthermore, the genes for three of the DNA methyltransferases have been cloned and sequenced (Table 2, appendix 3). In the past year we have isolated and characterized a second restriction and modification system (called M.CviAII and CviAII) in the prototype virus PBCV-1. M.CviAII methyltransferase methylates adenine in CATG sequences and CviAII endonuclease cleaves CATG, but not C^mATG, sequences. Thus CviAII endonuclease is an isoschizomer of the bacterial enzyme NlaIII, but unlike NlaIII which cleaves after the G, NlaIII cleaves between the C and A. The M.CviAII methyltransferase and CviAII restriction endonuclease genes have been cloned from virus PBCV-1

DNA and sequenced (Nelson, Zhang, Nietfeldt, Burbank, and Van Etten, manuscript in preparation). CviAII endonuclease is the first chlorella virus endonuclease to be cloned and sequenced.

3. Glycosylation of chlorella virus glycoproteins is unusual.

The prototype chlorella virus PBCV-1 contains three glycoproteins, including the major capsid protein Vp54. We have isolated a family of PBCV-1 mutants containing major capsid proteins with altered glycans. Typically, carbohydrate moieties of viral glycoproteins are added in the rough endoplasmic reticulum and Golgi apparatus by host glycosyl transferases. Viruses acquire their glycoprotein(s) by budding through a host membrane, usually the external membrane, as they leave the cell. Changes in the glycan portion of virus glycoproteins occur either by altering the viral host or by mutations in the peptide portion of the glycoprotein which change the glycosylation site. However, neither mechanism explains the changes in the glycan(s) of the PBCV-1 major capsid protein. All wild type and mutant chlorella viruses are grown in the same host and the predicted amino acid sequences of the major capsid protein from PBCV-1 and two of its mutants are identical (Wang, Li, Que, Lane, and Van Etten, manuscript in preparation). In addition, intact infectious PBCV-1 particles accumulate inside the cell prior to virus release; thus virus assembly is independent of release from the cell.

These results are explained most simply if PBCV-1 codes for a family of enzymes involved in its glycosylation (glycosyl transferases?). However, even if the explanation is more complex,

PBCV-1 has a novel method of producing glycoproteins. Whatever the mechanism, virus genes influence the glycan moiety.

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