Volume 17 Number 18 1989 Nucleic Acids Research

Nucleotide sequences of three Nodavirus RNA2's: the messengers for their coat protein precursors

Ranjit Dasgupta and Jean-Yves Sgro

Institute for Molecular Virology, University of Wisconsin, Madison, WI 53706, USA
Submitted August 4, 1989 EMBL accession nos X15959–X15961 (incl.)

Nodamura virus (NOV), Flock House virus (FHV) and Boolarra virus (BOV) are members of the family of insect virus called *Nodaviridae*. They are serologically distinct from each other. NOV was originally isolated from mosquitoes in Nodamura, Japan. The natural hosts for FHV and BOV were separate species of grass grubs from New Zealand and Australia respectively. All nodaviruses infect and grow well in wax moth larvae. FHV and BOV are routinely propagated and grown in cultured *Drosophila melanogaster* cells. NOV does not infect *Drosophila* cells. It is unique among the Nodaviridae in its ability to cause fatal infection in vertebrates (suckling mice, ref 1).

The genomes of Nodaviruses consist of two single stranded messenger sense RNAs contained in a single virion. RNA1 codes for the replicase function and RNA2 codes for the precursor for the capsid protein. Both RNAs have a 5'-terminal cap m7GpppGp and a blocking moiety, presumably a protein at their 3'-termini (2). We report here the complete nucleotide sequence of NOV, FHV and BOV RNA 2's derived from virion RNA's and their full length or near full length cDNA clones. Oligonucleotides used as primers at the 3'-end for the synthesis of single stranded cDNAs were designed from the known sequence of Black Beetle Virus (BBV) RNA2 (2) assuming a homology between these viruses. Transcripts derived from cloned FHV cDNA were infectous to cultured *Drosophila* cells (3). Sequences of the cloned cDNAs were determined from the fragments produced by restriction digestions and also after making nested deletions using exonuclease III, by means of the dideoxy chain termination method. 95 bases at the 3'end of NOV RNA2 and 60 bases at the 3'end of BOV RNA2 were also determined by direct enzymatic RNA sequencing of ^{32}P-labelled fragments produced by limited ribonuclease T1 digestion of virion RNA2. The initiator AUG codons for all these RNAs (underlined in Fig. 1) are very close to the 5'-termini. NOV, FHV and BOV code for 399, 407 and 403 amino acids respectively. NOV RNA2 has a direct repeat of 21 nucleotides (b 1235-1255 and b 1262-1282) near the 3'-end. Comparison of these sequences and also the published sequence of Black Beetle Virus RNA2 (2) shows that FHV and BBV are very closely related to each other (80% homology) whereas BOV and NOV are somewhat distantly related to BBV (50% homology).

```
N   1  GUAAACAACCAAUAACAUCA UGGUAUCCAAAGCAGCACGC CGCCGAAGAGCGGCCCCUCG ACAACAACAACGACAACAGU
O  81  CGAACCGUGCAUCAAACCAG CCUCGUCGGAGGAGAGCUCG CCGUACUCGAAGACAGCAGA GAAUGGCAGCAACAAACAAC
V 161  AUGCUGAAAAUGAGCGCUCC CGGACUCGAUUUUCUGAAAU GUGCCUUUGCUUCGCCGGAU UUUUCCACCGAUCCCGGCAA
  241  AGGUAUUCCAGACAAAUUUC AGGGUCUCGUUUUACCGAAG AAACAUUGUCUGACCCAGUC GAUAACGUUUACUCCGGGGA
R 321  AACAGACGAUGCUGUUGGUU GCACCUAUUCCUGGAAUUGC UUGCCUGAAGGCAGAAGCGA AUGUUGGCGCAUCCUUUUCA
N 401  GGUGUUCCUCUUGCCUCAGU UGAAUUUCCAGGGUUCGACC AGCUGUUUGGCACGUCAGCA ACUGACACCGCAGCUAAUGU
A 481  CACUGCUUUCCGGUAUGCGU CAAUGGCAGCCGGUGUUUAC CCCACGAGCAAUCUAAUGCA AUUUGCUGGGUCAAUACAGG
2 561  UGUACAAGAUACCUCUAAAA CAGGUUCUUAACUCCUACUC UCAGACCGUAGCGACCGUAC CACCUACCAACUUGGCUCAG
  641  AACACAAUUGCAAUAGAUGG ACUAGAAGCGUUAGAUGCAC UACCAAAUAACAACUACUCC GGUUCUUUUAUCGAGGGUUG
```

Figure. 1 Nucleotide sequences of NOV (top), FHV (middle) and BOV RNA2's (bottom). Start and stop codons are underlined. The sequence of one or two bases at the 3'-end of NOV and BOV RNA2 (indicated by an X) could not be accurately determined due to the presence of a blocking group.

References: (1) Bailey, L. & Scott, H. A. (1973) Nature, 241, 545.
(2) Dasgupta et al. (1984) Nucl. Acids. Res. 12, 7215-7223.
(3) Dasmahapatra et al. (1986) Proc. Natl. Acad. Sci. USA 83, 63-66.