Scheme of RNA transcription in calicivirus-infected cells

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Feline calicivirus (FCV) is a member of the Caliciviridae and contains a positive-stranded, non-segmented, polyadenylated RNA genome. It codes for at least one structural protein [1, 2] and one nonstructural protein [3]. The mode of synthesis of the viral RNA is still not clear. Neil and Mengeling identified three subgenomic RNAs in addition to the full length genome [4]. However, Carter reported that the number of virus specific RNAs detectable in infected cells was 8, and showed that RNA species 1 to 5 were represented as negative strands which may serve as templates in the synthesis of these RNAs [5].

Neil and Mengeling also reported the existence of a 3' co-terminal nested set of subgenomic RNAs in common with other virus families [4].

This communication describes work to further elucidate the scheme of RNA transcription in calicivirus-infected cells.

We have cloned and sequenced the entire genome of the FCV F9 strain [2]. The complete sequence is 7690 bases long and has two major open reading frames. Restriction fragments from different regions of the genome were used as probes (summarised in Table 1) and their position with respect to the FCV genomic RNA is presented in Figure 1.

Table 1. Location of probes.
a. Locations are expressed in number of nucleotides from the 5' end of the genomic RNA.

<table>
<thead>
<tr>
<th>Clones</th>
<th>Locationsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>p5E5</td>
<td>51 - 1182</td>
</tr>
<tr>
<td>J65</td>
<td>2407 - 3710</td>
</tr>
<tr>
<td>J66</td>
<td>3281 - 5649</td>
</tr>
<tr>
<td>Capsid gene</td>
<td>5313 - 7325</td>
</tr>
<tr>
<td>J67</td>
<td>7326 - 7690</td>
</tr>
</tbody>
</table>

Figure 1. Genomic location of probes that were used in northern blot analysis of FCV-infected cells.

Total RNA was extracted from FCV F9 infected CRFK cells at 5h post-infection, separated on a denaturing agarose gel, transferred to nitrocellulose filters by vacuum blotting and hybridised with each radio-labelled FCV probe.

It was found that the probe prepared from the 3' end of the genome hybridised with all of the FCV transcripts. As the probes used were progressively more 5' within the genome, there was a corresponding loss of hybridisation to the shorter subgenomic RNA (Fig. 2). This demonstrates that the RNAs are co-terminal, nested sets of transcripts in which the transcripts begin at specific points and then continue to the 3' end of genome. This pattern of transcription is similar to that employed by the coronaviruses and the togaviruses [6, 7, 8]. These findings also agree with the results of the preliminary study conducted by Neil and Mengeling [4]. Work is currently underway to determine the specific start point of transcription of the major subgenomic RNA.

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