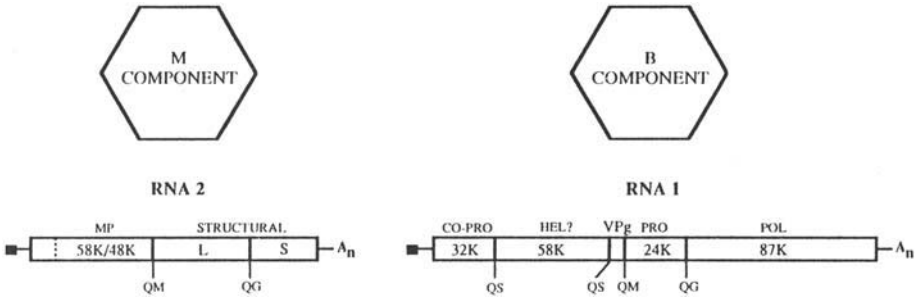

Fig. 1. Genetic organization of the CPMV genome. The icosahedral virus particles consist of two components, denoted B and M, that contain RNA-1 and RNA-2, respectively. The ORFs in the RNA molecules are indicated with open bars and VPg with a black square. The RNAs are translated into polyproteins that are proteolytically processed at six specific sites, indicated below the ORFs, into several stable intermediate and nine final cleavage products. These latter products are indicated in the ORFs. Functions of the different domains in the polyproteins are shown above the ORFs with the following abbreviations: MP, movement protein; CO-PRO, cofactor required for proteinase; HEL?, putative helicase; PRO, proteinase; POL, RNA-dependent RNA polymerase.

genomic RNAs of the comoviruses have a small protein covalently linked to their 5' end (denoted VPg: *V*iral *P*rotein *g*enome-bound) and a poly(A) tract at their 3' end (**Fig. 1**).

All comoviruses sequenced so far contain a single, long open reading frame (ORF) that occupies over 80% of the length of the RNA. Expression of both RNAs involves the production of large polyproteins, from which several smaller proteins are derived by proteolytic processing through the action of a viral proteinase (**Fig. 1**). The RNA-2 of all comoviruses tested are translated in vitro into two carboxy coterminal polyproteins, because of initiation of translation at a second in-frame AUG codon. For CPMV, this has also been shown to occur in infected cells. A total of 15 intermediate and final cleavage products have been identified in cells infected with CPMV as a result of processing at six specific dipeptide sequences (**Fig. 1**). CPMV RNA-1 is able to replicate independently from RNA-2 in cowpea protoplasts, and all proteins coded for by RNA-1 are needed for viral RNA replication. RNA-2, on the other hand, is needed for the infection of whole plants and specifies the two capsid proteins and the movement protein (**Fig. 1**).

The purification and extraction methods described below have been developed for CPMV. With minor modifications, these methods should also be applicable for other comoviruses (*see* **Subheading 4.**). The precipitation of