IN CELLULO versus IN VITRO RECONSTITUTION OF RNA-SPECIFIC VIRUS-LIKE PARTICLES

William (Bill) Gelbart

1. Abstract

The unique and huge advantage of in vitro reconstitution of specific-mRNA-containing virus-like particles (VLPs) is that the gene-delivery particle is perfectly monodisperse and that we can be sure that only two components are present – the purified capsid protein (CP), and the purified mRNA. In the case of CP from cowpea chlorotic mottle virus (CCMV) and of tobacco mosaic virus (TMV), this is possible for a broad range of RNA sequences and lengths. Preparing these pure reagents and carrying out their controlled co-self-assembly into gene-delivery particles, however, is expensive in time and money and cannot be easily scaled up for translational medicine purposes. Accordingly, we have designed a strategy for programming mammalian cells to synthesize these particles for us, taking advantage of the fact that packaging of RNA by TMV CP requires a particular nucleotide (nt) sequence – the Origin of Assembly Sequence (OAS) – in the RNA. The other device we exploit to ensure the specific in cellulo packaging of this OAS-containing RNA is to make it the only RNA molecule that is being replicated and that is therefore present in overwhelming numbers. More explicitly, we co-transfect cells with the following two RNA molecules: (1) an mRNA for a therapeutic gene of interest that is genetically fused to an RNA replicase gene so that the overall molecule, containing the OAS, is strongly replicated; and (2) an mRNA, not including the OAS, that encodes the TMV CP and that is replicated by the replicase gene product of molecule (1). In this way, a large amount of TMV capsid protein is synthesized in the cell, which packages molecule (1) essentially exclusively because it is the only RNA being replicated – and hence dominant in number – and because it is the only RNA including the OAS “packaging signal”.