Small RNA Plus-Strand RNA Bacteriophages

Examples: Qβ, MS2, R17, f2, fr

The small RNA bacteriophages have several interesting properties.

- They have the smallest genomes of any known virus, coding for only 3 or 4 gene products.
- DNA is not part of the life cycle. The basic molecular processes of DNA replication and transcription are not involved.
- These virus have a unique enzymatic activity, an RNA-dependent RNA polymerase, not ordinarily found in any cellular organism. This type of enzyme would have been the essential basis for replication in an RNA WORLD.

The main difference between the example in the book "MS2", and the example in the diagram below, is the presence of a discrete (overlapping) gene for a lysis protein in MS2. In R17 the lysis function is incorporated in a domain of the maturation protein (A).
**A (Maturation)**

- 330 amino acids
- 1 copy per virion
- Host recognition (pilus)
- Host cell lysis (inhibits MurA, first step of peptidoglycan synthesis)

**Coat Protein**

- 129 aa
- 180 copies per virion

**Replicase**

- 580 aa
- RNA-dependent RNA Polynmerase
- Assembles with 3 E. coli ribosomal proteins
  - Tu: Translational elongation factor; 393 aa binds aatRNA's
  - GTPase
  - Ts: Translational elongation factor; 282 aa GDP/GTP exchange
    on Tu
  - S1: 557aa 30S ribosomal subunit

The Qβ replicase has been used in some intriguing experiments in *in vitro* evolution and in a biotechnological application referred to as SELEX technology. SELEX is an application of the *in vitro* RNA replication to a "evolve a drug" strategy.

http://www.lmb.uni-muenchen.de/groups/famulok/SELEX.html

The 3 gene products of the R17 genome are needed in widely different amounts, and at different times during the infection cycle; (coat/Maturation 180/1). There being no transcription involved, regulation is at translational level. The ribosome binding site for translation of the A (maturation) protein is obscured by + strand secondary structure which only becomes accessible as the replicase unwinds the region. Replicase gene expression must not be done this way. Coat protein binds to ribosomal binding site #3 an reduces translation.

Translation of coat protein by host ribosomes. Initiation codons for Maturation protein and replicase are blocked by secondary structure.

Translation of coat protein gene exposes initiation codon for replicase gene.

Translation of replicase.

Replicase copies (+)-RNA into (-)_RNA.

Replicase copies (-)-RNA into (+)_RNA.

Initiation codon for Maturation protein is exposed in nascent (+)-RNA copy.

Coat protein binds initiation codon for replicase and represses translation.
RNA replication by Q beta replicase: A working model
Proceedings of the National Academy of Sciences of the United States of America
93: (21) 11558-11562 OCT 15 1996

Abstract:

Two classes of RNA ligands that bound to separate, high affinity nucleic acid binding sites on Q beta replicase were previously identified. RNA ligands to the two sites, referred to as site I and site II, were used to investigate the molecular mechanism of RNA replication employed by the four-subunit replicase. Replication inhibition by site I- and site II-specific ligands defined two subsets of replicatable RNAs. When provided with appropriate 3' ends, ligands to either site served as replication templates. UV crosslinking experiments revealed that site I is associated with the S1 subunit, site II with elongation factor Tu, and polymerization with the viral subunit of the holoenzyme. These results provide the framework for a three site model describing template recognition and product strand initiation by Q beta replicase.

A persuasive model that justifies the presence of host proteins EF-Tu and S1 in the QB RNA Replicase.

Both host factors are involved in template recognition rather than polymerization per se. EF-Tu binds the 3' end of the + strand because it is AC-rich, similar to the acceptor stems of tRNA's (the "normal" ligands for EF-Tu). S1 binds to pyrimidine rich tracts as found at the 3' end of the QB - strand RNA. (Presumably the "normal" ligand of S1in the ribosome is a pyrimidine tract in rRNA?).

This model lends little support to the wild speculation that protoribosomes were capable of RNA replication as well as translation.

Gold's paper is worthwhile reading as a gateway to SELEX technology.