CHAPTER 3

STRATEGY OF REPLICATION OF THE VIRAL GENOME

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Abbreviations:

ss = single-strand(ed); ds = double-strand(ed); (+) = nucleic acid strand with the same sequence as mRNA or anti-sense DNA; (−) = nucleic acid strand with the same sequence as sense DNA (or RNA equivalent); bp = base pair(s).

INTRODUCTION

Unlike higher organisms, viruses utilize a wide range of strategies in the replication of their genome. This is clearly a consequence of their need to deal with genomes that may be single-stranded (ss), double-stranded (ds), or partially ds; with genomes that may be composed of DNA, RNA, or both (at different stages in their life cycle); and with genomic structures which may be simple circles, simple linear molecules, single-stranded linear structures with terminal hairpin folds, ds linear structures in which the complementary strands are covalently linked at the ends, multipartite structures, and complex linear structures. In some cases the genome may also include covalently-linked proteins.

The variability of viral genomes (particularly the presence of RNA and single-strandedness) is probably an example of the selective influence of size and complexity in evolution. As a genome becomes larger and more complex, there is a greater need to minimize strand scission and to prevent copying errors in order to minimize the frequency of lethal defects per genome. Just as one cannot obtain man-sized insects because of inherent limitations in insect structure at large sizes, one probably cannot have a genome as complex as *E. coli* (or even vaccinia) composed or RNA or which is single-stranded. Evolution favors the more chemically stable DNA and

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the more structurally stable ds forms of genomes as genome size increases. Indeed, the larger single-stranded RNA viruses resort to segmented genomes (1, 2) to circumvent some of these difficulties.

Given the extreme variability of virus structures, it would be unrealistic to expect a unified mechanism of replication. However, there are common problems which all viruses must solve during nucleic acid replication; problems which are a consequence of polymerase enzymes which require a template, initiating factors (or primer in the case of DNA viruses), and the fact that all known polymerases can only add 5'nucleotide triphosphate to the 3' end of a growing chain.

After a general analysis of how viral replication is studied, we will examine the general problems and properties of replication of RNA viruses (Section 1), DNA viruses (Section 2), and viruses with both RNA and DNA forms (Section 3). In each section, we will discuss replication in specific selected viruses and bacteriophage. We will make particular note of the production of virus-specified replicative enzymes and the formation of viral, host, or mixed enzyme complexes, the mechanism of initiation (or priming) of replication, the formation of replicative structures and, finally, how the virus completes replication so as to end up with a full-length viral genome.

Viral One-Step Synchronized Growth Cycle

Before examining the molecular mechanism of viral replication one needs to standardize certain fundamental parameters of infection such as the type and physiological state of the host cells and the most suitable media and serum concentration for optimal viral production. One of the most important parameters affecting studies of viral replication is the multiplicity of infection (m.o.i.). Too high a m.o.i. can cause cell lysis from without, and too low a m.o.i. may result in undetectable levels of viral RNA or DNA at crucial early stages.

Before starting any studies on viral nucleic acid synthesis, it is important to carry out a single-step growth experiment in order to have a time-frame for further experiment. Typically, one wants to synchronize viral infection for all cells of the population to simplify analysis of viral products at various times during the infectious process.

An idealized one-step growth cycle for synchronized infection demonstrates several distinct stages in the infectious process. The first stage is attachment of the virus to a receptor site and penetration of the virus into the cell, or in the case of phage, the introduction of the nucleic acid into the bacterial host. Once this has been accomplished, and unattached virus removed, infectious particles are not usually detectable, even in the cell. This second stage is termed the *eclipse period* and ends with the earliest detection of infectious *intracellular* virus. This period should be distinguished from the *latent* period, the time at which extracellular virus is first released from the cell. From the end of the latent period, we see a large increase in the number of virus particles formed. This period is termed the growth period and is the time of maximum viral assembly, maturation, and release.

Starting the virus-cell interaction with a large m.o.i. (10-100) ensures a synchronous infection. After washing off unadsorbed virus, and if necessary adding

anti-viral antibody to prevent a further round of adsorbtion and replication (which should not be a problem with cytolytic viruses introduced at a high m.o.i.), samples are assayed at various times (usually by plaque assay) for infectious material. Any free virus present early in the infectious process is typically due to unadsorbed virus.

Viral RNA or DNA synthesis is normally measured by labelling the cells with radioactive nucleic acid precursors, either continuously throughout the cycle, or in short pulses. In the case of many RNA viruses, one can also use an inhibitor of host-dependent RNA synthesis, such as actinomycin D, to analyze viral RNA synthesis independent of host RNA synthesis. For DNA viruses, the size of the viral DNA or specific hybridization probes can be used to distinguish viral DNA from host DNA. In some cases (e.g., T-even phages) one can use the presence of unusual nucleotides to identify viral DNA.

1. RNA VIRUSES

The most salient features of the replication of RNA viruses is that RNA viruses lack unwinding and ligating enzymes and must replicate in an assynchronous fashion from the viral ends. They cannot replicate *both* strands from an internal initiation site, nor replicate in a discontinuous fashion. Two general types of RNA replicative structures can be found:

i) the replicative intermediate (RI), which includes a single-stranded template with several newly-made transcripts peeling off (Fig. 1A), and
ii) the replicative form (RF), which involves the formation or utilization of a double-stranded structure (Fig. 1B).

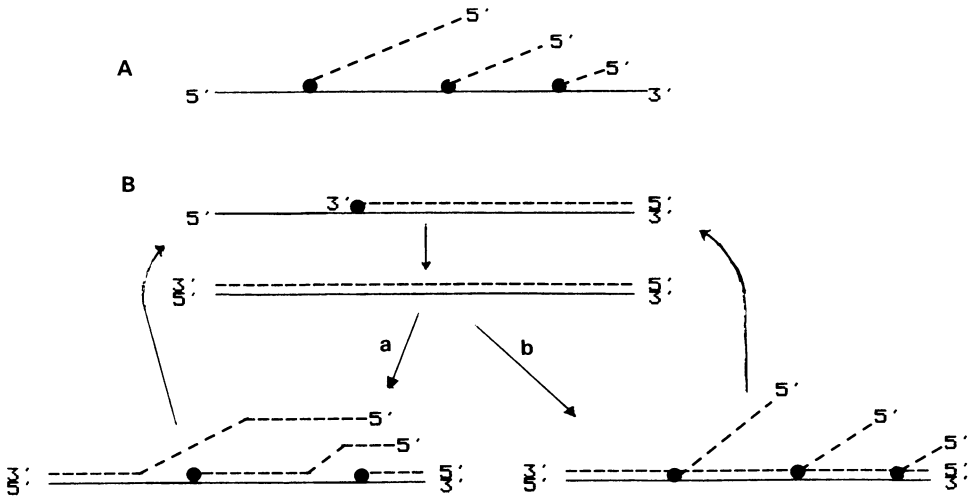

Figure 1. Mechanisms of RNA Replication. A. Replication via a replicative intermediate (RI). B. Replication via a double-stranded replicative form (RF). a. Semiconserative replication of RF. b. Conserative replication of RF.

The replication of RF can proceed either by semi-conservative displacement or in a conservative manner to produce ssRNA (+) strand, (-) strand, or mRNA. Note that the semi-conservative displacement replication of RF differs from RI only in the initial presence of ds RNA and in the extent of hybridization between newly-synthesized and template strands. Thus ds RNA viruses or RF structures produce displaced single strands which subsequently serve as template for formation of the complementary strand (3).

In general viral RNA replication exploits features of host transcription/translation systems rather than host replication systems. Specifically, some RNA viruses utilize tRNA-like structures (4, 5, 6), host translation factors (7, 8), host 3' polyadenylation enzymes, 5' capping enzymes, and even 5' fragments of host mRNA (9-14) in the replicative or transcription process. Viroids, the only RNA virus-like replicative entity which do not rely on viral replicase (plant virus satellites (15) do), rely on host DNA-dependent RNA polymerase (16, 17).

With the exception of a very few covalent circular single-stranded RNA species — not viruses but viral satellites — replication starts at the viral ends. However, many RNA viruses have strong intra-strand hydrogen-bonding giving a complex secondary structure typically having short hairpin or stem-loop structures. Some viruses have complementary ends or include proteins which interact to create a non-covalently closed circle. In many cases, the full-length virion and/or antivirion RNAs are always encapsidated.

The RNA replication/transcription mechanism must, at minimum, accomplish two discrete tasks. It must:

i) generate full-length genomic RNA apt to be encapsidated, and
ii) secure the synthesis of trnaslatable mRNAs.

Except for picornaviruses and the small RNA-containing phages (in which genomic RNA is identical with mRNA) *replication* (i.e: the synthesis of full-length genome-like RNA), and *transcription* (i.e: synthesis of viral messenger RNAs) are two totally distinct processes that take place at different times and under different conditions. The mechanism(s) that trigger the shift from *transcription* to *replication* will be discussed in detail in the following chapters. For the purpose of this review it is important to stress that in order to produce full-length genome-like RNA the replication machinery must generate a full-length anti-genomic strand and transcribe the template so generated into RNA copies of genomic polarity.

The requirement for complete viral information in both strands can be circumvented if there are non-template-directed mechanisms for regenerating complete viral information on the strand(s) which is/are to be packaged as virion (7, 21). In the case of multipartite viruses, the individual RNA segments typically use the same basic replication mechanism (not necessarily the case with contaminating satellite species) and can be treated as minor variations of unipartite genomes for purposes of examining the replication process. Multipartite genomes have more importance as a *transcriptional* mechanism than as a distinctive *replicative* one. Because of the highly assynchronous nature of RNA replication, one must examine the synthesis of (+) and (-) full-length transcripts separately, since the 3' ends of

the (+) and (−) strands can (and do) differ in some viruses, thus requiring (in some cases) different initiation mechanisms.

In addition, because viral (−) strands are also used as the template for mRNA synthesis, a third task is often imposed on the replicative system, the production of mRNA.

Usually, the mRNA is distinctive from the full-length (+) transcript. Often the mRNA is subgenomic (initiated internally, terminated early, or both) or the mRNA may utilize distinctive 5' ends (e.g., by loss of VPg in picornaviruses, or the presence of host-derived 5'-capped sequences as in orthomyxoviruses or bunyaviruses). Often there is a sequential nature to mRNA, (−) strand, and (+) strand transcription, with a switch from greater mRNA production to greater virion transcription as infection proceeds. The distinctive nature of mRNA may play a role in preventing its encapsidation and directing it into polysomes.

In principle, RNA replication of both strands can proceed from the precise 3' end of the template strand. Since only a single virus-encoded RNA-directed RNA polymerase is present in RNA viruses, other factors (host- or virus-derived) are usually involved in directing the polymerase to initiate at the 3' end of (+) or (−) template, to produce mRNA, and, in some cases, not to terminate early during full-length (+) strand synthesis.

A. Positive-strand RNA Viruses

This category includes the bacteriophage group Leviviridae (e.g., Qβ, MS2, f2, R17). Animal (+) single-stranded RNA virus groups include the Caliciviridae, Picornaviridae, Alphaviridae, Flaviviridae, Coronaviridae, and Nodaviride. And most plant viruses are (+) single-stranded RNA viruses. These viruses exhibit considerable variation in the initiation of synthesis of (+) and (−) strands, often, but not always, using different mechanisms for each strand.

Qβ phage replication and tRNA-like ends

In addition to phage-specified core replicase, the complete replication complex of Qβ phage requires:

i) the 30S ribosomal S1 protein (22) complexed to replicase,
ii) the protein elongation factors EF-Tu and EF-Ts (23) acting as a EF-Tu.Ts complex, and
iii) another host factor protein (24) needed for initiation. This factor has single-stranded RNA-binding activity, and the level of factor determines the ratio of (+) to (−) strand synthesis.

The complete replicase complex recognizes several regions of the Qβ RNA sequence or structure (7) and binds, in the presence of GTP and Mn^{+}, to the 3' end of phage RNA, a 3' CCCA-OH sequence. Transcription of the (−) strand starts with a GTP opposite the penultimate C and ends with a C opposite the 5' terminal pppGGG of the (+) strand. At this point a termination event adds a 3'-terminal A (25, 26) in a non-template-directed fashion. A similar process occurs in the replica-

tion of (+) strand RNA, thus regenerating the terminal A lost during (-) strand replication. The non-templated terminal A may be added by the replicase since *in vitro* replication systems produce full-length virus.

Despite the nominal correspondence of the viral end to the 3' termini of tRNAs, which can have their 3' ends completed post-transcriptionally by tRNA nucleotidyltransferase, neither the replicase (7) nor tRNA nucleotidyltransferase (27) can add a terminal A to *free, complete* (+) or (-) strands which have had their terminal A removed. This may reflect an altered secondary structure of isolated complete strands (compared to newly-synthesized strands associated with replicase). Endonuclease-cleaved phage (as opposed to intact phage) treated with snake venom phosphodiesterase can have terminal CCA added to two specific fragments by tRNA nucleotidyltransferase-including what appears to be 3' terminus (27). Regardless of the possible enzymatic roles of the host factors in replication, it is evident that recognition of tRNA-like structures (presumably specific stem-loop structures) plays a role in assembly of replicase complex at the appropriate site.

The 3' ends of a number of plant (+) strand RNA viruses have a tRNA-like structure (21). These structures interact with host tRNA-related enzymes. Indeed, in some cases, the packaged virion, in at least part of the population, lacks the terminal A, which can be added post-infection by tRNA nucleotidyltransferase (21). The plant virus tRNA-like 3' structures, unlike the RNA phage 3' end, can also be aminoacylated by the appropriate tRNA aminoacylsynthetase (21). In analogy with the binding of EF-Tu and EF-Ts by RNA phage, the aminoacylated (but not the unacylated) tRNA-like structure binds EF-1 (4, 6, 21) in the presence of GTP. Recent analysis of the ability of the tRNA-like end of brome mosaic virus to recognize viral replicase or tRNA aminoacylsynthetase indicates that the recognition of viral end by replicase is affected by deletions in arm B and in the anticodon of arm C (Fig. 2). Base substitutions in the anticodon loop also causes loss of replicase-binding activity without affecting aminoacylation (29). In contrast, aminoacylation is affected by deletions of arm B in a manner generally proportional to its effect on replication (with some exceptions which affect aminoacylation without greatly affecting replication) but is not greatly affected by deletions in arm C. These plant viral terminal structures can form alternate secondary structures (4, 6, 21), which may also play a role in replication.

The ability to produce specific mutations, produce full-length viral RNA sequences (30), and to create *in vitro* replication systems (31) should help to resolve some of the details of the replicative role of tRNA-like struc in the near future. Of particular interest will be the differences in the replication complexes used in producing (-) antivirion and (+) virion.

Picornavirus replication and the role of VPg

Picornaviruses, using poliovirus (see chapter 8 and ref. 32, 33, for reviews) as an example, are single-stranded (+) RNA viruses which are polyadenylated at the 3' end. The 5' end is linked to a short peptide (VPg), which, however, is not essential for infection (34, 35). Other VPg-containing (+) RNA viruses, however, do re-

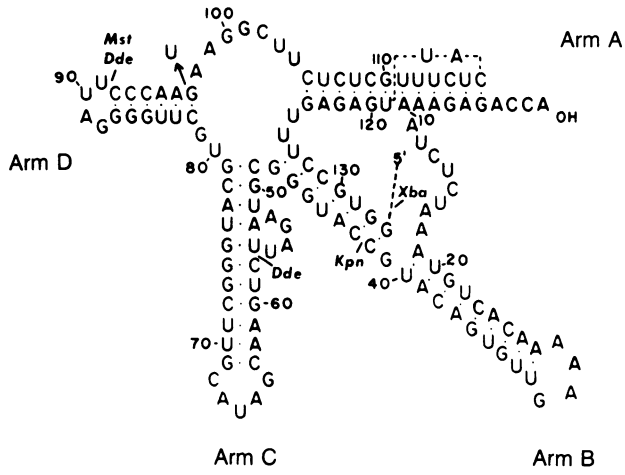

Figure 2. The 3' tRNA-like structure of brome mosaic virus. The role of arms B and C are described in the text. Reprinted with permission (reference 28).

quire VPg for infectivity. Although VPg-containing poliovirus RNA can act as mRNA *in vitro* (36) and are sometimes found in polysomes late in replication (37), most (+) strands found in polysomes end in 5'-pUU-3' and have had the VPg removed (34), presumably by a host enzyme found in uninfected cells (38). This enzyme has an unknown normal cellular role. The level of this enzyme and coat protein during the course of infection may play a role in regulating the fate of the (+) transcript. In contrast to the non-essential nature of VPg for initial infectivity, removal of the poly(A) tail greatly reduces infectivity (39).

The virion (+) RNA is mainly translated from an internal AUG as a polyprotein which is proteolytically cleaved to generate non-structural and structural proteins (chapters 5 and 8 of this volume, and ref. 32, 33). Among these peptides are the virally-encoded RNA-dependent RNA polymerase (40) and VPg.

The first step in viral replication, the synthesis of (-) antivirion from (+) virion, can be performed *in vitro* and requires 2 or 3 proteins: the viral polymerase, a host factor recently identified as a terminal uridylyl transferase (41, 42), and possibly, a VPg donor. Recent evidence (43-45) suggests that VPg is *not* required *in vitro* for initiation of (-) strand of the virion (+) strand.

Two models of replication have been proposed: according to the first one, the host factor would add an oligo-U sequence to the poly-A tail, leading to hairpin formation. The hairpin would then be extended by viral replicase. *In vitro*, this results in a 2-unit length fold-back genome with VPg attached only to the 5' end of the original (+) virion template. Since, *in vivo*, the 5' end of the (-), antivirion strand has VPg (46, 47), the hairpin, if formed *in vivo*, must be quickly cleaved, presumably with addition of VPg (see Fig. 3A). VPg, in such a model, might play a role in preventing the formation of ds RNA — the RF (replicative form) which has been considered a dead-end product.

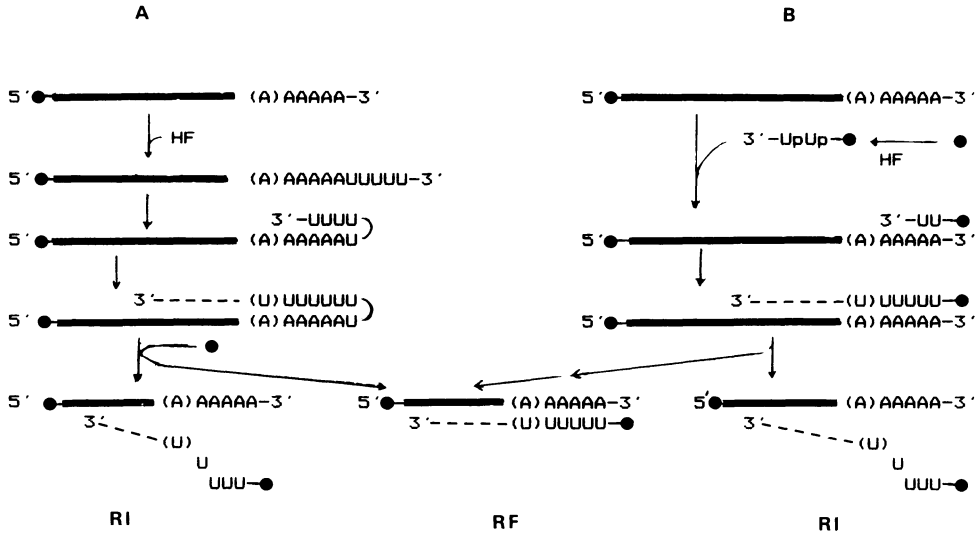

Figure 3. Two models of picornavirus replication. A. Replication via hairpin formation. B. Replication via use of VPg primer.

Most nascent virus RNA is in the form of replicative intermediates (RI) with the template being transcribed by multiple replicases (32, 33).

In a contrasting model of replication, VPg (or precursor) can itself be uridylated to VPg-pUpU (47, 48). Although this has not been chased into longer RNAs (47), it could act as a primer for either (-) or (+) synthesis (50), providing an alternative model for (-) strand replication (see Fig. 3B). At the present time, it is not possible to distinguish between these models.

Replication of (+) strand starts with a 5' UU sequence, which is highly conserved in all picornaviruses (Fig. 3). The 5' sequence is different from the complement of the sequence at the 3' viral (non-poly(A)) end (51). This, of course, may explain why poly(A) is necessary for replication. The 3' viral (non-poly(A) ends are conserved only within genera (51). However, using cloned cDNA, a small insertion in this 3' non-coding region produces a *ts* mutant for productive infection (52) indicating that these sequences are important. The importance of the natural 5'-UU sequence to virion production can be seen from *in vitro* synthesized rhinovirus RNA. The RNA produced *in vitro* has an additional 21 nucleotide 5' of the natural 5' end. This RNA is infectious, but when the progeny are recovered, the natural end has been regenerated, despite the presence of a second UU in the 5' added sequence (53). A similar process may occur in virus produced by transfection of cloned DNA copies of poliovirus (54). The regeneration of natural viral 5' ends is compatible with either model of picornavirus replication.

The role of VPg proteins in the replication of plant and other animal (+) single-stranded RNA viruses is even less clear, although in some cases the VPg is

necessary for infection (51). Similarities to picornaviruses do exist in plant viruses (55).

Clearly, one must be cautious about ascribing a priming role to VPg proteins. RNA polymerases, unlike DNA polymerases, do *not* require a stabilized 3'0H primer and *can* start synthesis directly at the 3' end of a template. However, doing so usually involves more than the core polymerase itself, and one mechanism of initiation could involve a priming protein. In such a model, the difference between those viruses in which VPg is necessary for infection and those in which it is not may be similar to the distinction between adenovirus and Φ29 bacteriophage, the linear DNA viruses that utilize priming proteins (see Section 2). The recent development of cloned poliovirus, which can be used to produce poliovirus RNA (56), will allow the production of specific mutants which may resolve some of these questions.

Alphaviruses, Flaviviruses, Coronaviruses, and timing of (-) and (+) strand synthesis

Alphaviruses, Flaviviruses, and Coronaviruses have radically different mechanisms for transcription of mRNAs off of the anti-genomic, (-) strand.

Alphaviruses produce a single subgenomic mRNA (in addition to a genome-length (+) strand which acts as virion RNA *and* mRNA). The genomic RNA is directs the synthesis of a polyprotein encoding 4 nonstructural proteins, the last of which requires read-through of a stop codon (51) for translation. Both (+) strand RNAs are capped and have poly(A) (chapter 9, and ref.51).

Flavivirus (+) RNA, in contrast, is capped but not polyadenylated (51). *No subgenomic mRNAs are produced.* Rather, like picornavirus, a polycistronic protein is produced which is cleaved during translation in such a way that the polyprotein never actually has an independent existence (57). Little is known about flavivirus replication.

The genomic RNA of Coronaviruses is capped and polyadenylated and a nested series of subgenomic mRNAs has been identified in coronavirus-infected cells.

All of the mRNAs have the same 5' leader sequence (58-60), which apparently primes the nested subgenomic mRNA species. All the genomic and subgenomic (+) mRNAs terminate at the 3' terminus of the virion RNA (51) and are polyadenylated. Only the 5'-most open reading frame of each mRNA is translated (51).

However, these disparate viruses may replicate by similar mechanisms. In each case, the 3' end of the (-) and (+) strands, upon which the replicase complexes must be assembled, are quite different (51, 61, 62), both in sequence and in secondary structure (62), allowing temporal regulation of (+) strand, (-) strand, and mRNA synthesis.

We will examine the replication of alphaviruses, especially Sindbis virus, in detail. A model has been proposed (63) whereby the preferred template for replication is the (-) strand, with preferential initiation of (+) strand occuring at the internal conserved subgenomic mRNA site (51). Typically, (-) strand production oc-

curs early in infection in both alpha (64-66) and coronaviruses (67), and is later shut off or greatly reduced. Late in infection, the rapid inclusion of full-length (+) strands into capsids may deplete available template for (-) strand synthesis. Recent work (68) with a cloned defective interfering (DI) particle of Sindbis virus shows that only sequences in the 5'-most 162 nucleotides and 3'-most 19 nucleotides of the DI are needed in *cis* for its replication and packaging.

The 3' end of the DI is the same as that of the native virus, and is strongly conserved in all alpha viruses (69) and natural DI particles.

The 5' end of this particular cloned DI, like many Sindbis DI particles generated in chicken embryo fibroblasts (but not baby hamster kidney cells) (70,71) have nucleotides 10-75 of cellular $tRNA^{asp}$, in this case replacing the 5'-most 30 nucleotides of native viral sequences. The common occurrence of $tRNA^{asp}$ in DIs from these cells may imply a role of tRNA aminoactylsynthetase in replicase assembly, perhaps by recognition of a viral template structure. The recognition need not be specific for $tRNA^{asp}$, if this tRNA is the only one whose complement permits replicase assembly.

In this respect, it is of interest that viral 5' *sequences* are not strongly conserved among alphaviruses (62), but a *structure* (which would also be present on the 3' end of the template (-) strand) appears to be conserved. Such a complex betweeen 3' template structure and host aminoacylsynthetase may, occasionally, put an uncharged tRNA in a position to be (aberrantly ?) extended by replicase.

Other DI particles have either native 5' ends, 5' ends which have undergone sequence rearrangement, and even one which has 100 nucleotides of the 5' end of the subgenomic mRNA added to the native 5' viral end (70). Clearly the ability to assemble replicase at the 3' end of antivirion RNA does not require a specific sequence, nor (given the ability of rearranged 5' ends to function) does replication require, necessarily, that recognition structures be positioned precisely at the 3' end of the template. This does not mean, however, that there is much flexibility at the 5' end: removal of even 7 nucleotides from the 5' end of the cloned $tRNA^{asp}$-containing DI eliminates its ability to replicate.

Another strongly conserved region in alphaviruses and their naturally occurring DI particles is a 51-nucleotide sequence (62) seen at 155-205 bases from the natural viral 5' end. This sequence can be removed in *in vitro* constructed DI particles without eliminating replication and packaging, implying that the sequence is *not* required in *cis* in avian cells. The sequence may, however, have a quantitative effect or play a role in the arthropod host. Little is known about replication of these viruses in their arthropod hosts.

Sindbis virus has two complementation groups in which temperature-sensitive mutations affect production of (-) strands:

i) complementation group B contains a temperature-sensitive mutant, *ts*-11 (65), whose normal product is required for production of (-) strands; and

ii) complementation group A including a second group of temperature-sensitive mutants (e.g: *ts*-24), whose active product is necessary for shutting off (-) strand replication. The assignment of complementation groups to specific polypeptide products is incomplete.

The group A product is probably not acting as a protease which inactivates the B product, since shifting to the non-permissive temperature late in replication in the absence of new protein synthesis results in (-) strand synthesis, indicating that functional B product still is present.

Since both the (+) genome-length RNA and the subgenomic mRNA include the same 3' sequences, and no truncated (-) strands are seen, the (-) strand replicase complex must recognize both the 3' and 5' end of the (+) strand simultaneously in order to assemble the replicase enzymes only on full-length (+) RNAs (51), presumably aided by the possibility that the virion, *in vivo*, may be present as a non-covalently linked circle (72). Perhaps B product is involved in directing the elongation factor — the presumed core polymerase (complementation group F) — to the 3' end of (+) strand full-length virion. Complementation group A product, then, may subsequently block binding of polymerase to the B product. Synthesis of the (-) strand starts within the poly(A) tail and produces a poly(U) 5' sequence on the (-) strand (73), which, like all (-) RNA strands is uncapped. Extensive production of (-) strand requires continued protein synthesis, indicating that one of the replication complex factors is unstable (64). Production of (+) strands (both virion and subgenomic mRNA) does not require continued protein synthesis (74).

Coronavirus replication also exhibits a time-dependent reduction of (-) strand synthesis, although the reduction is not as sharp as in alpha viruses. Here, also, synthesis of (-) strand is more sensitive to inhibition of protein synthesis than is (+) strand synthesis or mRNA synthesis (67), and the polymerase complexes involved in replication of (-) and (+) strands may differ from each other (75, 76).

In these viruses, the role of host factors is unclear but may be of major importance in assembling the replicase complex on either virion or antivirion RNA. Any use of host factors by alphaviruses, however, must be compatible with the divergent eucaryotic organisms (both arthropod and vertebrate) within which these viruses replicate.

The absence of similar 5' and 3' structures or sequences at the virion ends points out that replication recognition signals need not be the same at the 3' end of (-) and (+) templates, and that these differences can be exploited to time the production of virion RNA, mRNA, and antivirion RNA.

B. Negative-strand RNA Viruses

The replication of negative-strand, single-stranded RNA viruses can be considered in two distinctive groups:

i) the unipartite viruses, rhabdoviruses and paramyxoviruses, in which the process of transcription and replication are clearly distinct: in addition to producing a full-length anti-genomic (+) transcript which is uncapped and not polyadenylated (*replication*), the virus transcribes a number (5-7) of monocistronic mRNAs which either initiate internally or are processed to an internal start site and which typically terminate prior to the 5' end of the virion RNA (51, 77);

ii) the multipartite negative-strand single-stranded RNA virus groups orthomyxoviruses, bunyaviruses, and arenaviruses in which, in general, each RNA segment produces a single monocistronic mRNA (although a few RNA segments, via mRNA splicing, proteolytic processing, or via an ambisense RNA segment which encodes sense information for one gene at the 3' end of the (+) strand and sense information for a second gene at the 3' end of the (−) strand, do produce more than one protein).

The latter group of viruses — with the possible exception of arenaviruses, about which little is known (78) — utilize capped host mRNA 5' fragments as primers for transcription of their mRNA. The mRNA terminates at a polyadenylation site near, but not at, the 5' end of the template (−) strand. In contrast, the (+) strand full-length template is not capped, starts without host primer, is encapsidated like the (−) virion, and does not terminate at the polyadenylation site, but proceeds through to the end of the (−) template (77, 79).

In both types of virus, then, the (−) template is used to produce two distinctive products, capped and polyadenylated subgenomic mRNAs and uncapped, unpolyadenylated full-length (+) transcripts.

All of the negative-strand RNA viruses, unlike many positive-strand RNA viruses, contain complementary or nearly complementary sequences of between 10-20 nt at the extreme 3' ends of (+) and (−) templates indicating similar recognition signals for replicase assembly on both strands. In the case of the multipartite viruses, the 3' terminal sequences are very similar on the different segments (51, 77, 79).

However, other sequences at the 3' end of the templates probably play a role in both the switch between mRNA production and (+) strand synthesis and in the switch from producing more or less equal amounts of (+) and (−) strands to producing primarily (−) strands (virions) late in infection.

Rhabdoviruses and Paramyxovirus

The rhabdovirus Vesicular Stomatitis Virus (VSV) and the paramyxoviruses Sendai and New Castle disease virus (NDV) are cytoplasmic and do not require continued cellular mRNA synthesis in order to transcribe viral mRNA (80, 81). VSV is quite capable of replication in an enucleated cell, whereas the paramyxovirus require a nucleus for virion production, although the nucleus need not function biochemically (81). Transcription and replication of VSV (see Fig. 4) occurs

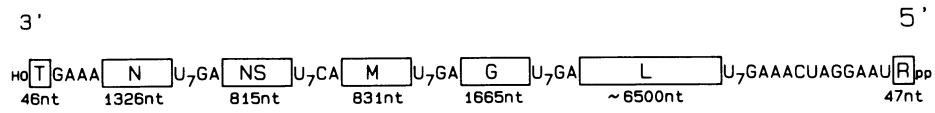

Figure 4. Structure of VSV. T and R represent the terminal leader sequences of (−) and (+) virion, respectively. N, NS, M, G, and L represent mRNA sequences of viral genes. Each mRNA is capped, starts at the 5' end of each box and ends within the oligo (U) sequence at the 3' end of each box. Reprinted with permission (reference 51).

on an RNA-N protein complex. Transcription requires NS and L proteins, both of which are required for polymerase activity. NS protein, but not L protein, can bind to the RNA-N protein complex (82). NS protein can be phosphorylated and the degree of phosphorylation — high (NS2) or low (NS1) — may influence whether replication or transcription occurs (51, 83, 85), perhaps related to the influence of pH (86, 87). Phosphorylation and dephosphorylation appears to be accomplished by host kinases and phosphatases.

Two other proteins have been implicated in viral replication:

i) the M structural protein, which may, by interacting with nucleocapsid at the 3' region of the (-) strand, prevent substantial transcription off of the (-) strand. *In vitro*, its presence permits synthesis of only the leader and 14 nt of the N gene. It may also, by helping to package the (-) strand, prevent continued synthesis of (+) template (88); and
ii) the N protein is also involved in replication since neither full-length strand is present as unencapsidated RNA.

It is clear that continued protein synthesis is necessary for VSV *replication* but not for *primary transcription* (89). This may reflect a requirement for N protein to bind the (+) strand leader (89), or it may reflect a need for newly-synthesized (altered ?) NS protein.

A model for N protein-mediated regulation has been proposed (91) which accounts for the role of the leader sequences in transcription and replication. Basically, it is proposed that both replication and transcription initiate at the extreme 3' end of (-) or (+) strands. This transcript is not capped, and transcription continues through to a decision point (45-47 nucleotides in the case of (+) strand leader synthesis (92, 93), 46 or 50 nt in the case of (-) strand leader synthesis (94).

At this point, some signal — either the level of N protein (91), the degree of phosphorylation of the NS protein of the replicase (51, 83, 87), or perhaps interaction with host protein (93, 93, 95) — will result in one of two choices:

i) there will be either termination (or possibly, processing) of leader transcript and re-initiation (or processing) of what is now mRNA transcription 4 nucleotides downstream from the termination site (96). This new mRNA transcript is capped (presumably, by viral enzymes); or
ii) transcription will continue through this decision point resulting, eventually, in full-length transcript.

To produce full-length (+) antivirion transcript, however, replicative transcription must continue through several internal transcription termination sites. In contrast, mRNA transcripts terminate at these signals (or are processed at them) and transcription usually, but not always, restarts 2 nucleotides downstream of each termination site. Each new mRNA transcript is capped and a polar effect is observed, with 5'-most transcripts present at higher levels than 3'-most transcripts. The mechanism which prevents termination in full-length transcripts is not known. Perhaps the presence of N protein on (+) replicative RNA or the presence of cap on mRNAs signal whether the replicase will proceed through these termination signals. Anti-termination, of course, could also represent a property of

differentially-modified NS protein or even an indirect effect, such as changed cell pH. The (+) replicative RNA proceeds through the final mRNA transcription termination/polyadenylation site to the end of the (-) template and is not polyadenylated. It is also uncapped—although the 5'-terminal γ phosphate is usually removed to leave a ppA 5'-terminus.

In producing (-) virion RNA from full-length (+) antivirion RNA, it is proposed that a similar process occurs, since the inverted terminal repeats at the ends of the viral strands present the replicative machinery with similar end structures. Here also, the replicase appears to pause after producing a short leader sequence. However, signals indicating termination of nearly synthesized (-) strand are rare, although termination does occur. Typically, then, full-length (-) strand transcription continues.

This model would be strengthened if N protein were found to bind processively and co-operatively to RNA from a signal sequence within the 5' leader. Replication of the paramyxoviruses is probably quite similar (51, 77, 97) given the similarities in genome sequence, gene order and transcriptional processes.

Orthomyxoviruses and Bunyaviruses

The replication of segmented orthomyxoviruses (chapter 13) has been recently reviewed (14, 77) and, like the other negative-strand RNA viruses, the (+) antivirion RNA and (-) virion RNA are always present as nucleoprotein, in contrast to the mRNAs.

The mRNAs also differ in sequence from the (+) antivirion RNA. In the case of influenza (orthomyxoviruses), the virion contains has 8-9 RNA segments. The 3' ends of each of the segments are largely identical to each other within the first 13-22 nt, and very similar to the 3' ends of the (+) antivirion strands (Fig. 5), differing primarily in the presence of different nucleotides at position 3, 5, and 8 (98). These conserved sequences are probably involved in the recognition of the 3'-ends by the transcriptional enzyme complex. There are at least 3-4 proteins involved in the transcription/replication complex. Two of these, PB1 (P1) and PA, probably represent the core polymerase functions, required for both transcription and replication (19). A third protein, PB2 (P3), forms a complex with PB1 and PA during transcription (99). It is unclear whether PB2 plays a role in replication (100). The fourth protein, NP, is the nucleocapsid and complexes both (+) antigenome and (-) virion, with the mRNAs remaining unencapsidated.

Influenza virus transcription, and probably replication, occurs in the nucleus (101). Transcription/replication occurs early during infection (102) and cannot occur in the absence of continued host mRNA synthesis (81, 103). The PB2 protein acts as a cap-binding protein which binds new host mRNA and cleaves it 10-14 nucleotides downstream of the cap, preferentially after a purine (12, 104). This host mRNA stretch bound to PB2 then becomes complexed to the PB1 and PA proteins. The host mRNA oligonucleotide is positioned such that its 3' purine (typically an A) is opposite the 3' terminal U on the (-) template. The PB1 protein then binds GTP, complementary to the penultimate C on the (-) template (104) and polymerization proceeds. After initiation, the complex of all three proteins,

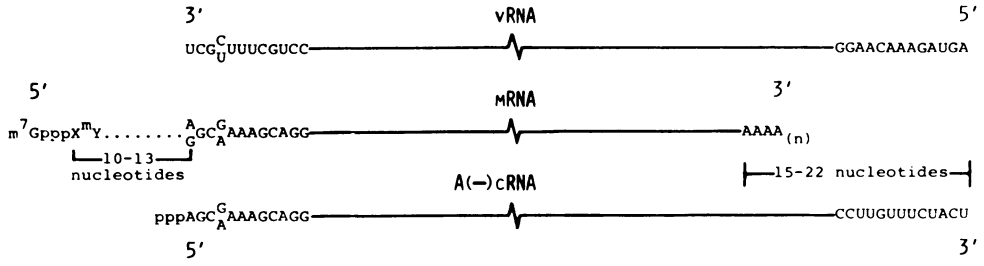

Figure 5. 5' and 3' sequences of influenza virus virion RNA, antivirion RNA, and mRNA. Reprinted with permission (reference 14).

with the cap still associated with PB2, continues transcription for at least 10 nucleotides before the 5' end dissociates from PB2. The replication complex continues until it reaches a tract of 5-7 U residues located about 20 nucleotides from the 5' end of the template. At this position, the capped influenza mRNA terminates (105) adding variable amounts of poly(A). The result is an mRNA transcript which includes non-template 5' sequences and lacks the final 15-22 nucleotides of (−) template (see Fig. 5).

In contrast, *replication* of both (+) and (−) full-length strands initiates directly at the 3' end of the template (HO-UpCp) leaving a triphosphate at the 5' end (pppApG). *Replication* does not terminate at the poly(U) stretch, but proceeds through to the end of the template 15-22 nucleotides downstream.

It is possible that nucleocapsid binding may play a role both in antitermination and, perhaps, in determining whether transcription or replication is initiated. There is essentially no knowledge of the mechanism by which (−) strands are preferentially exported or by which the different segments are appropriately packaged together.

The replication of bunyaviruses is probably, to a large extent, similar to that of the orthomyxoviruses (79). These viruses have a tripartite genome, the segments of which are rarely packaged in an equimolar fashion. The 3'- ends of (+) and (−) full-length transcripts are quite similar for both the S and M subunits of Snowshoe Hare bunyavirus (107) and the two segments also have considerable sequence homology at their ends.

The mRNAs of both the S and M fragment utilize host-derived 5'-capped oligonucleotides of 12-17 nucleotides to prime mRNA synthesis. Both terminate mRNA transcription before the 3' end of template — about 100 nucleotides before in the case of S segment, about 60 nt in the case of the M segment (107). Lacrosse virus has been shown to have a primer-stimulated RNA polymerase and a methylated cap-dependent nuclease (101). Full-length transcripts start precisely at the 3' end of template, are present as nucleoprotein and proceed through the mRNA termination sites. Unlike orthomyxoviruses, however, bunyavirus replication/transcription is cytoplasmic and does not require continued host mRNA synthesis. Presumably the source of host 5'-capped oligonucleotides is preformed host mRNAs (79).

In contrast to the many positive-strand RNA plant viruses, there are only two groups of negative-strand RNA plant viruses: plant rhabdoviruses and tomato spot wilt virus, which has been suggested to be a bunyavirus (109). There are no known negative-strand RNA bacteriophage.

C. Reovirus and Double-stranded RNA Replication

When reovirus enter lysosomes, the virus particle becomes uncoated with consequent activation of the double-stranded RNA-dependent RNA polymerase (110). The intermediate subviral particles probably then enter the cytoplasm where transcription occurs.

The virus contains 10 double-stranded RNA segments (111) which appear to be perfectly base-paired with no single-stranded RNA tails (112), and which are capped at the (+) strand 5' termini (113, 114).

Transcription, like replication, starts at the precise 3' end of a (-) strand and proceeds to the 5' end of template (115). No poly(A) tail is present. Only the (+) strand is transcribed off of ds RNA, and transcription is fully conservative (116) with neither parental strand appearing among the transcription products. Elongation, rather than initiation, appears to be rate limiting. Transcription from different RNA segments, each of which encodes a single message, does not all occur simultaneously, some segments being transcribed early and others later (117).

Replication proceeds initially like transcription, by conservative transcription of a capped (+) strand off the parental ds RNA template which remain in a subviral particle with active viral capping enzymes. Eventually these capped transcripts become associated with a new subviral particle where (-) strand synthesis occurs. The initiation, but not elongation, of (-) strand synthesis off of the (+) template in the new subviral particle requires protein synthesis (115). The newly-synthesized (-) strand remains associated with the (+) strand to form a new ds RNA molecule (118). Only a single round of (-) synthesis occurs on each (+) template (119). Like transcription, it appears that those (+) strands destined for the replication of certain of the ds segments appear earlier than those of other segments (120).

In contrast to the conservative replication seen in reoviruses and in the noninfectious ds RNA mycovirus of *Saccharomyces cerevisiae* (121), replication of the ds RNA bacteriophage Φ6 (122) and other mycoviruses (123, 124) appears to be by a semiconservative displacement mechanism (see Fig. 1B).

D. RNA Circles: Viroids, Virusoids and Plant Satellite Viruses

Although covalently closed circular genomes and multimer production are common features of many DNA viruses (see Section 2 of this review), they are only rarely seen in RNA. Indeed, they are mostly seen in plants — the delta "virus" associated with hepatitis B infection is an exception — and are associated with small satellite viruses, virusoids, and viroids (15, 16, 19, 125). These RNA elements typically encode few, if any, obvious proteins, but utilize either viral RNA-directed RNA polymerase (required for virusoid and satellite virus replication) or host DNA-directed RNA polymerase (capable of being utilized by viroids).

Multimeric RNAs of Satellite Tobacco Ring Spot Virus (STobRV) (126, 127), potatoe spindle tuber viroid (PSTV) (128), and possibly some virusoids (129) have the apparent ability to generate (in some cases spontaneously) monomeric subunits which are 5' HO-Ap and 3' C-2',3' cyclic phosphodiester (126-128). The monomer can, in some cases, also spontaneously dimerize (or circularize) due to the nature of the cyclic phosphodiester — the number of phosphoester bonds is unchanged in linear monomer, circular monomer, and dimer forms (126, 127). In other cases, RNA ligase may be involved in circularization (128, 129). That the most abundant form of STobRV are linear monomers whereas that of viroids is circular monomers may be irrelevent to their replicative scheme, since host cells may contain activities (such as RNA ligase) which permit, assist, or catalyze circularization (128-130) or oligomerization (131). One can envision production of multimeric or circular (-) strands off a circular (+) template by a rolling circle model, which, in turn, can act as a template for production of multimeric (+) strands. The multimeric (+) strands can then (possibly autocatalytically) produce circular or monomeric products. Alternatively, oligomerization might, in some cases, proceed in the absence of circularization by processes similar to splicing (131), producing 5' phosphates and 3'-OH linear monomers.

These subviral RNAs are invariably small and exhibit very high intrastrand complementarity. These features may be requirements for replication, cleavage, and ligation of these RNA molecules and may limit the ability of such RNAs to code for proteins. This may limit the use of a rolling circle or multimeric replication strategy in RNA to non-informational parasitic molecules, despite the obvious enzymatic capabilities of RNA molecules (132, 133).

One should note the similarity of some of these processes to those which are involved in processing tRNA precursors. The autocatalytic (132) or RNase F-assisted (134) cleavage of a tRNA-like T4 RNA (or of tRNA precursor) depends upon a stem-loop structure and produces a 5'-OH adenine and 2',3' cyclic phosphodiester C, as is the case in PSTV (128) and (+) strand of STobRV (126, 127). These ends can be annealed by RNA ligase, which normally acts on tRNA precursors, to initially produce a CpA dinucleotide in which the C has a 2' phosphate in addition to the 3',5' phosphodiester linkage to A. This unusual nucleotide is seen in some virusoids (129).

The complementarity of the (-) strand of PSTV to U1 RNA, discussed in the preceding chapter, (16) and the intron-like features of peanut stunt virus associated RNA 5 (131) may mean that other (splicing-derived?) features may be involved in the production of cyclic, monomeric, or oligomeric forms of these replicative RNA molecules.

E. RNA Replication: A Summary

RNA virus replication studies have (and will) be greatly aided by the recently acquired ability to clone DNA copies of the viral (or subviral) genome and produce RNA transcripts in vitro. This allows one to explore the structural and sequence requirements for replication in a precisely defined way. One is also struck by the extensive utilization of host factors and processes which normally utilize tRNA

molecules and by the extent to which "ends" play a role in RNA replication. With the exception of circular RNAs, replication (as opposed to transcription) starts at one end of a virus and proceeds to the other. In many cases, the virus or its mRNA is capped, and this is true of those viruses which are cytoplasmic as well as those which are nuclear, indicating that the capping enzyme activity may be viral in origin in some cases. Only (+) strands are capped, and presumably 5' sequences (structure ?) of full-length and subgenomic (+) RNAs are important in determining whether the sequence is capped.

2. DNA VIRUSES

DNA viruses differ from RNA viruses in that they need not encode their own polymerase (although many of the larger ds DNA viruses do), in that many can integrate into the host genome (retroviruses (see Section 3), of course, do so as a double-stranded DNA), and in that recombination can play a role in replication. In general, whereas RNA viruses exploit host translation/transcription proteins as associated factors in their replication, DNA viruses exploit host DNA replication enzymes in their replication.

No known DNA polymerase, unlike RNA polymerases, initiates synthesis on a template in the absence of a stabilized primer providing a nucleotide 3'-OH end.

Typically, this primer is a short ribonucleotide synthesized by an RNA polymerase. Replication of ds DNA is always semi-conservative, like the replication of host DNA. Moreover, the presence of host and viral DNA ligases, topoisomerases, and recombinational enzymes — including transposases — provide a repertoire of alternative structures not seen in RNA replication, perhaps because such enzymes are not seen in transcriptional/translational processes.

The fact that DNA polymerases cannot initiate at the extreme 3' end of a linear template poses problems for the replication of *linear* DNA viruses: how to replace the 5'-most oligoribonucleotide primer with DNA, particularly in the absence of any enzyme (host or viral) capable of adding template-directed nucleotides to the 5' end of an oligonucleotide ? The structural mechanisms by which DNA viruses solve this problem will provide the basis for organizing the following sections.

A. Simple Circles: Papova Viruses

The simplest way to avoid problems of replicating the ends of a DNA molecule is not to have ends, but to replicate via a circular molecule. The simplest model is that of the papova viruses. These viruses are small ds DNA circles which in the nucleus are typically coated with about 24 host-derived nucleosomes containing histones H2a, H2b, H3, and H4 but not H1 (chapters 18 and 19, ref. 135).

The replication complex is largely host-derived; the only virally-encoded protein(s) required for replication are the multifunctional T-antigen (T-ag) proteins. The T-ag proteins are expressed early in viral replication. The large T-ag is found primarily in the nucleus (136) and is the only protein absolutely required for the lytic cycle, although polyoma middle and small T-ag may enhance replication in

some, but not all, cell lines (137). T-ag can: 1) Stimulate G0/G1 arrested cells to enter S phase (138-140), thereby inducing the production of the host DNA-polymerizing system needed for viral replication. Induction of S-phase may be due to T-ag activity as a DNA-independent ATPase which is associated with a protein kinase activity (141, 142) or to its ability to bind to host p53 protein (143, 144). 2) Act as the primary regulatory molecule switching the ds DNA virus between early strand mRNA transcription, late-strand mRNA transcription, and initiation of replication, as a consequence of T-ag concentration and specific binding to several sites near the origin of replication (145-148).

Virtually all DNA replication in SV40 initiates within a 60-bp sequence almost entirely within T-ag binding site 2, and initiation requires the binding of T-ag to this site (149). Recent work indicates that the DNA between the pentanucleotide T-ag recognition sequences, to which the dimeric T-ag binds, is "bent" (150). A similar "bent" structure is seen at the λ origin of replication (151). *In vitro* studies (152) indicate that the interaction between bound T-ag and host replication complex is species specific and probably contributes to host-range limitations of the virus. The replicative role of T-ag other than initiation is not clear (153) although there is some evidence that it may remain associated with the replicase complex (154).

The leading (5' to 3') strand initiates with an oligoribonucleotide of 5-8 nucleotides starting with a ribo-A (155). Usually replication proceeds bi-directionally from the origin, with the 3' to 5' template strand being filled in by discontinuous synthesis in the retrograde (relative to fork movement) direction, in a manner similar to synthesis of other ds DNA molecules initiated internally.

Replication appears to pause after about 90% of the template duplication has occurred (156). This is an indication of a rate-limiting step at the final resolution of the two circles, perhaps a requirement for resolution of concatenated dimers (157). Duplex SV40 circles can be produced in in vitro replicative systems (158).

Many bacteriophage (both single-stranded phage in their double-stranded replicative forms, and double-stranded phage) also utilize this simple circular replication mechanism early in infection. Some later switch to a rolling circle mechanism. λ is, of course, a prime example (159).

B. Modified Circular Replication: Rolling Circles and Concatamer Formation

The bacterophage ΦX174, perhaps the most thoroughly analyzed virus in terms of its replicative scheme, is the model for rolling circle models of replication (see Fig. 6). Indeed, because of the extensive use of host enzymes, this phage has been used as a window to explore host replication (160-162).

The first step in replication of ΦX174 is the formation of a preprimosome complex, starting with host n, n', and n'' proteins.

n' binds to a specific 55 nucleotide fragment of phage single-stranded DNA which can form a 44-nucleotide hairpin structure. In the process of binding, ATP is hydrolyzed. The n' protein is capable of using the energy of ATP to propel the final primosome along DNA.

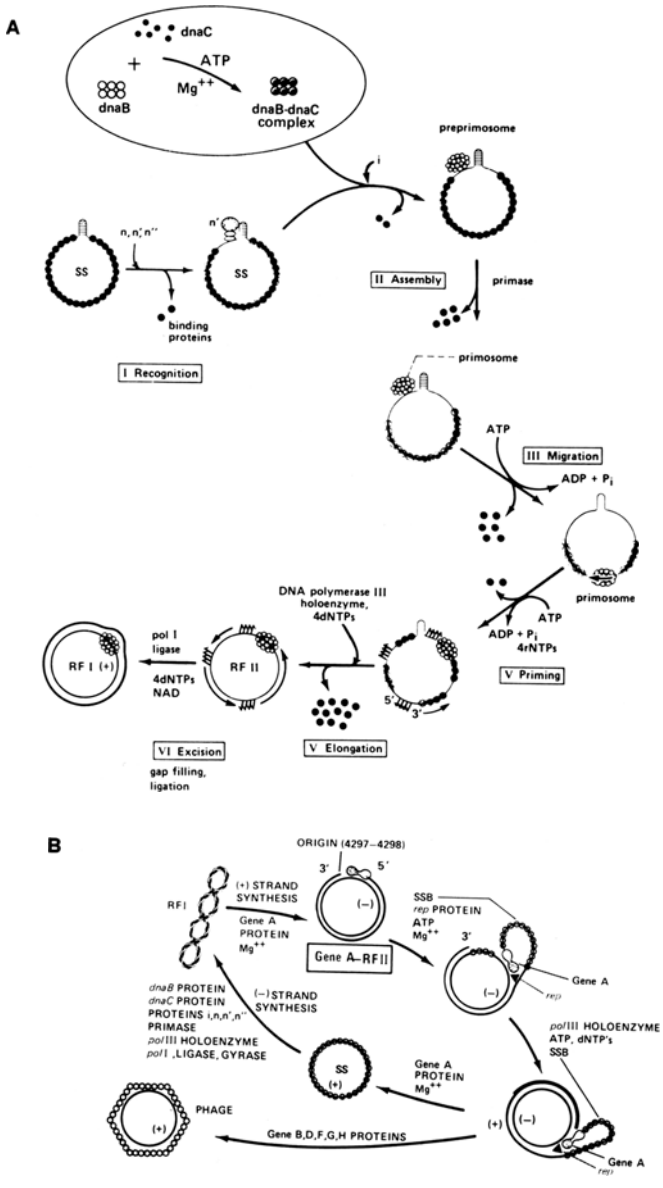

Figure 6. Replication of ΦX174. A. Formation of RF. B. Replication of RF to produce single-stranded virion. See text for discussion. Reprinted with permission (reference 160, 161).

Protein *n* serves to aid binding of dnaB protein, helping to form an initial complex containing *dnaB/dnaC* complex and *i* proteins to form the *preprimosome.*

Primase, the specialized RNA polymerase used for DNA initiation, is then added, forming the primosome, which can migrate to random sites on the ΦX174 genome to start DNA replication. Once a short oligoribonucleotide is synthesized, DNA polymerase activity proceeds, and, in conjunction with RNase H activity and ligase, produces a ds DNA circle — the replicative form (RF). At this point, viral

transcription off the (−) strand occurs, producing the important multifunctional viral A protein (162).

The A protein can cleave the RF (+) strand at a unique site in the hairpin structure. The nick provides a 3'-OH end upon which DNA polymerase can proceed, displacing the old strand. The similarity to a replication fork is obvious (see Fig. 6) and, indeed, only synthesis in the retrograde direction is needed to produce a ds linear concatameric structure like phage λ. The viral concatamers, however, may never actually form. After one round of replication protein A typically cleaves unit length (+) ΦX174. Because the cleavage site is within the hairpin structure, a ds region with a nick can form. The ligase activity of protein A can subsequently seal the nick to produce circular (+) virion. This scheme, differing only in detail, accounts for the replication of filiamentous single-stranded DNA circular phages, many ds DNA phages and, perhaps, for porcine circovirus, a circular single-stranded DNA animal virus.

A similar mechanism, circularization and concatamer formation by rolling circle replication has been suggested for herpesvirus replication (163, 164). However, the herpes system is more complex because these viruses undergo extensive recombination, particularly in repeat regions. When the repeats are in an inverted orientation, recombination results in packaged viruses which are isomers of the original virus with different orientations of the unique sequences between the inverted repeats (165). When the repeated sequences are direct repeats (especially terminal direct repeats) packaged virions often differ in the iteration number of the repeats (165). Since recombinational mechanisms can also produce concatamers (see Section 2 C.), recombination may also be involved in herpesvirus replication.

The *geminiviruses*, one of the few DNA plant viruses, contain two single-stranded DNA circles per genome. The two single-stranded circles contain a 200-nucleotide common region (the rest of the sequence of each circle is unique). The common region can form a hairpin with a GC rich stem (166, 167). Moreover, the packaged virion of one strain of the virus, Maize Streak virus (MSV), contains a small segment of complementary strand which has a few ribonucleotides at its 5' end and which terminates after 80 or so nucleotides. Other geminiviruses (e.g: Cassava Latent Virus) do not appear to have this short complementary strand. *In vivo*, after infection, one finds primarily ds circular molecules, dimeric concatamers, and occasional concatameric single-stranded DNA. Although not proven, the most likely mechanism of replication is via a rolling circle. The short second strand in MSV may be an "Okazaki" fragment which can act as a primer for RF ds circle formation or as a signal for processing the concatamers or a signal for packaging the virion DNA (these are not, of course, mutually exclusive possibilities).

C. Concatamerization by Recombination: T-Phage and Iridoviruses

T4 phage is probably the best studied virus which emphasizes a recombinational strategy for concatamer formation (168). T4 only rarely forms circles, but is packaged as a terminally-redundant permuted circle. Fig. 7 shows how T4 produces long branched concatamers. After initiation at one of several possible origins,

replication proceeds bidirectionally until the end of the linear molecule is reached. Because of the inability of DNA polymerases to initiate at the exact 3' end of the template DNA, this 3' end remains single-stranded . Such a single-stranded end is highly recombinogenic and can invade and displace the complementary region in another co-infecting phage, at the other end of a progeny molecule, or (rarely) at the other end of the same molecule producing a circular molecule. In any case, the invading strand provides a 3' end on a template molecule and is, in essence, a new replication fork (see boxed area in Fig. 7). As replication and/or branch migration continues, resolution of the branched structure, perhaps during the process of packaging, can occur by standard recombinational processes. The generation of replication forks by recombination is well-suited to rapidly accelerate overall DNA synthesis.

Recombinational processes may also play a role in the replication of herpes viruses, and may explain the finding that newly-synthesized and packaged virus contains a high proportion of nicks and gaps and explain the appearance of viral DNA in "tangles" (which have some similarity to the dense branching structure of T4) late in infection (163).

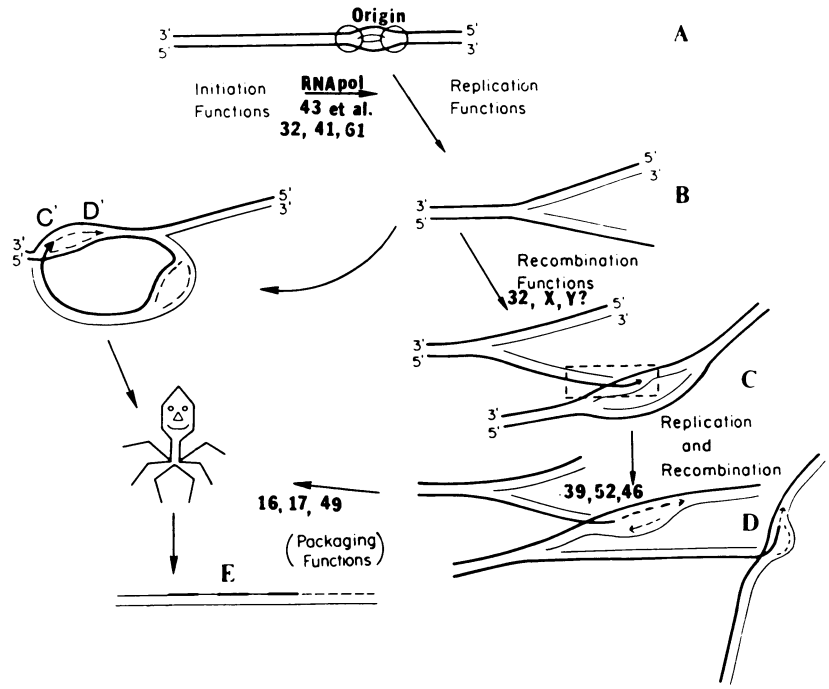

Figure 7. Replication of phage T4. A. Bidirectional replication from origin. B. Fork with single-stranded 3' end. C. Invasiveness of single-stranded end to form a new replicative fork (see boxed area). D. Further replication E. Resolution and packaging. Numbers and symbols in bold face indicate viral genes involved in that function. Reprinted with permission (reference 168).

The replication of the iridoviruses (169) is even more similar to T4. Iridoviruses are linear, terminally redundant, and circularly permuted (although, like phage P22 (170) the circular permutation is limited to certain regions — accounting for 20-28% — of the genome). Like many phage (e.g. ΦX174, λ) which replicate in two stages, an early stage producing monomeric RF and a later stage producing concatameric DNA, iridoviruses exhibit a staged replicative system. Early replication is nuclear and the size of replicating DNA is up to twice genome size. Late replication (>3hrs.) is cytoplasmic and produces DNA greater than 10X genome length (171). In addition to the displacement in time and space, the late-replicating DNA (but not the nuclear DNA) is heavily methylated (172) at the C position: apparently every CpG is methylated.

Another class of viruses in which recombination may play an important role are the poxviruses (discussed in detail chapter 21 and in Section 2 D. of this review). These viruses replicate entirely within the cytoplasm of the host in foci called *factory areas* (173), even in the absence of host nucleus (174, 175). The ability to produce recombinant viruses by site-specific recombination (176, 178) shows that recombination can, and does, occur in the cytoplasmic factory areas (presumably using viral enzymes). The extent to which recombination plays a role in poxvirus replication is unknown, although one model of viral replication involves recombination (179) as a means of resolving the termini. Concatameric DNA is observed (180, 181), but is consistent with other models of replication (180) as well.

D. Hairpin Structures and Viral Replication

The hairpin mechanism of dealing with the ends of linear DNA molecules was first proposed by Cavalier-Smith (182). Basically, for single-stranded linear DNA molecules, the end must be capable of folding-back to generate a self-priming hairpin structure with a 3'-OH end.

For linear ds molecules it is proposed that the end structure has complementary strands which are covalently linked at their ends upon which an endonuclease can generate a 3'OH end and a 5' hairpin. After polymerase activity, the terminal palindromes generated can reform into two terminal hairpins. This appears to be the mechanism which is used not only by the single-stranded DNA parvoviruses, and, possibly, the ds DNA poxviruses, but also by eucaryotic telomeres, e.g., in yeast (183).

Parvovirus replication

Two classes of the small (less than 6000 nucleotides) single-stranded linear DNA parvoviruses exist: the adeno-associated viruses (AAV) and the autonomously-replicating parvoviruses (ARPV). Since the AAV (184) and ARPV (185) exhibit significant differences in their replication process, they will be considered separately.

AAV requires co-infection with adenovirus or herpesvirus for productive infection. In the absence of the requisite helper functions from adenovirus or

herpesvirus, AAV can integrate into host chromosomal DNA as a full-length or nearly full-length provirus (see Section IV. F). Rescue of the latent AAV requires adenovirus, and, specifically (in addition to other regions required for replication) requires a functional product located within the EIB region of adenovirus (186, 187). In addition, AAV replication appears to require:

i) the EIA region of adenovirus, which encodes early functions (188, 189) that have a cascade effect on other early adenovirus functions. Whether EIA acts directly (188) or indirectly via other adenovirus functions is unclear; and
ii) the E4 region (190, 191) appears to be required for full replicative activity. (3) The E2A and VA1 regions appear to be related to AAV capsid mRNA production (192) and have an effect on the level of single-stranded AAV virion produced but not on the level of ds RF (193). This implies a role of capsid protein in the regulation of the switch from RF to virion production.

The E2B region, which encodes adenovirus terminal protein and polymerase, is not vital for AAV replication. This does not mean that these proteins play no role in AAV replication — AAV replication complexes isolated from herpesvirus co-infected cells contain herpes polymerase (194) — only that host proteins can be utilized as well.

The sequence of AAV (195) shows a 5' and 3' terminal sequence which contain two inverted repeats imbedded in a longer inverted repeat (Fig. 8). This sequence can fold into a "T"-shaped structure and can provide, probably through an intermediate circular structure (184, 196), a 3' end which can act as a primer for synthesis of a ds hairpin RF. The existence of these structures (and others) *in vivo* has led to the model of AAV replication seen in Fig. 8 (197).

Briefly, after the ds hairpin RF is formed, it is nicked opposite the site of initiation. This nick provides a second 3'-OH end which can act as a primer for polymerase to act on, producing a non-hairpin, full-length ds-RF. The site of cleavage preferentially gives a 3'-GTT-5' sequence at the 5' end of the ds-RF. However, a substantial percentage of virions end in 3'-GT-5' or 3'-G-5', with these microdeletions (and even larger ones) being regenerated upon RF formation (196-198). The ds-RF intermediate can then, presumably under the influence of DNA binding proteins, restructure its terminal segments to generate hairpin structures on both strands. This rearranged ds-RF provides a 3'-OH end which can act as a primer for replication. Replication of ds-RF results in displacement of a single-stranded virion DNA, which is presumably — at least late in infection — packaged by capsid proteins. A ds hairpin RF is also generated and it can be cleaved again and extended to regenerate full-length ds-RF.

One consequence of such a model is that there is a "flip-flop" of terminal sequences, and virion DNA — both (+) and (-) strands are packaged in AAV — will have 4 different isomeric orientations of its ends. This prediction is, in fact, confirmed in viral preparations (199).

The ARPV (Fig. 9) differ from AAV in structure (200). The 3' end is similar in structure (but not sequence) to that of AAV, but does not undergo "flip-flop"

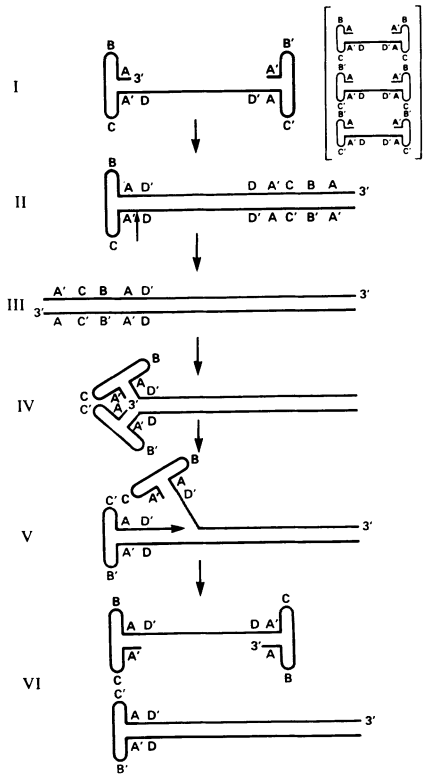

Figure 8. Replication of adeno-associated virus. See text for discussion. Reprinted with permission (reference 161).

isomerization, either in the packaged virion DNA or the several RF structures one can isolate (201). The 5' end of ARPV forms an imperfect simple hairpin which is different in structure and sequence from the 3' end. The 5' end is present as isomeric "flip-flops" in virion DNA and RF structures. Unlike AAV, typically only the (-) strand is packaged in virions (185). In addition, at least some of the RF 5' ends contain a covalently attached protein (of unknown, possibly host (202), origin) linked to an ~18-nucleotide extension beyond the viral 5' end. Again, the viral 5' end is not at a specific nucleotide but can be 1 or 2 nt shorter, indicating that the nuclease activity producing this end is probably cutting at a specific distance from a recognition structure rather than cleaving at a specific sequence. The extra 18 nt seen in some RF structures are viral in origin (201) and are removed before viral DNA is packaged. A model of ARPV replication, based on these constraints and the existence of appropriate intermediates has been proposed (185, 201) and is described in Fig. 9.

Replication of the ARPV requires concomitant host DNA synthesis (203) and appears to involve host DNA polymerases α and γ (204). It has been suggested (205) that production of ds RF involves polymeraseα, with polymerase γ — which is used in displacement replication of mitochondrial DNA in the cell (206) — being responsible for the displacement synthesis of virion DNA.

The source(s) of the three endonuclease activities required for the proposed replication model (one which cleaves the 5' hairpin 18 nt past the original viral se-

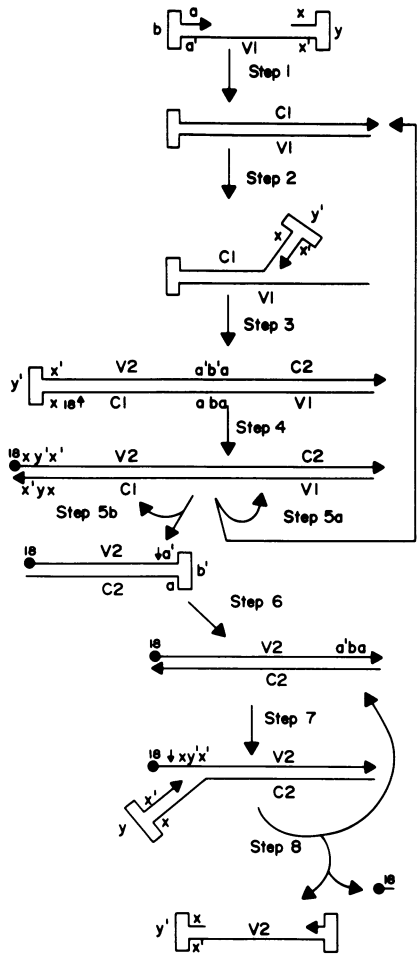

Figure 9. Replication of autonomous parvovirus. xyx' represents viral 5' and aba' represents viral 3' sequences. Solid circle represents 5' terminal protein. Reprinted with permission from *Virology* B.N. Fields, ed. Raven Press.

quence when present as a ds hairpin RF; one which cleaves the 3' hairpin only when it is present in the proper orientation relative to virion sequence, thereby avoiding isomerization; and one which removes the extra 18 nucleotides and terminal protein from the virion) are not known. Because both AAV and ARPV require structural (conformational) changes in the terminal regions involving melting duplex DNA to permit hairpin formation, DNA binding proteins are required and several strongly-(but not covalently) binding proteins of unknown, but probably host, origin (202) are found associated with RF structures.

Poxvirus replication

Poxviruses, unlike parvoviruses, replicate entirely in the cytoplasm utilizing viral enzymes for their replication. The viral DNA is nominally double-stranded, but both termini form covalently-linked imperfect hairpin structures which are pre-

sent as isomeric inverted complements in progeny viruses (206, 207) — i.e: they flip-flop like the terminal structures of parvoviruses. In addition, the similar inverted terminal regions at either end of vaccinia include two sets of a 70-bp tandem repeat, the first set of 13 repeats starts 87 base pairs from the proximal end and is separated by 325 base pairs from a second set of 19 tandem repeats. This is followed by a somewhat overlapping pair of 125-bp repeats followed by 8 tandem 54-base pair repeats.

All the repeats have regions of homology (208-209). There is variation of the number of repeats both within isolates of the same strain and between vaccinia and rabbitpox (205) and some sequence divergence between cowpox and vaccinia (210).

Replication appears to initiate near the termini of the virus and involves discontinuous synthesis with RNA primers (211-213). Whether a specific viral origin of replication exists in poxviruses is not known, since, unlike other viruses (214-216), replication of plasmids introduced into cells co-infected with vaccinia does not require the presence on the plasmid of a specific viral origin of replication or, indeed, any viral sequence at all (217).

Nicks are introduced near the end of parental viral genomes soon after infection (218, 219) and are only sealed late in infection (220). This has led to the proposal that these nicks provide a 3'-OH end, which, when extended, provides a terminal hairpin palindromic sequence (see Fig. 10). When these ends are restructured

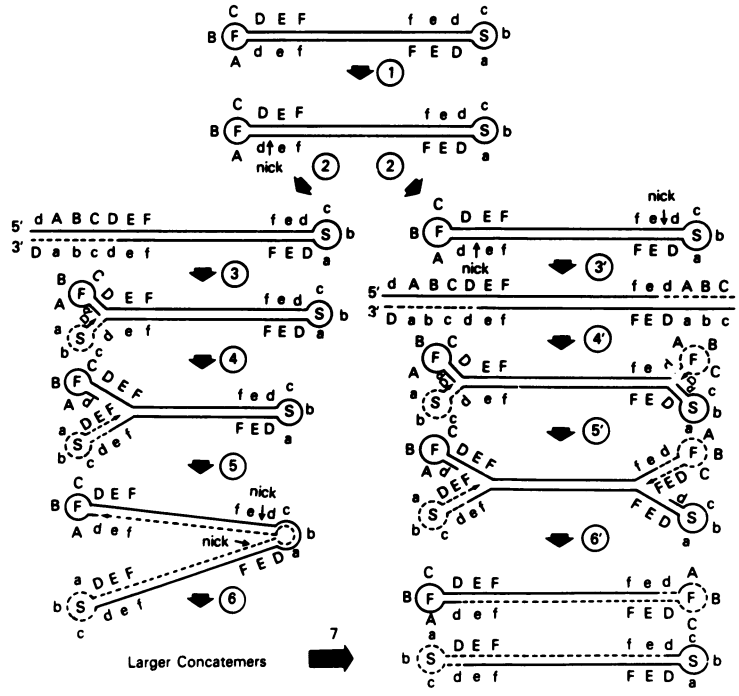

Figure 10. Poxvirus replication. S and F represent isomeric forms of termini. See text for discussion. Reprinted with permission (reference 180).

into two terminal hairpins, one of the hairpins provides a 3'-OH end which can act as a primer permitting synthesis from the resolved ends (205, 207, 221-223). Note that retrograde synthesis on the lagging strand of resolved vaccinia termini produces a ds replication fork and fills in single-stranded gaps.

Although vaccinia termini can be attached in vitro to a linear yeast plasmid, these ends apparently do not act as telomeres in yeast. DNA synthesis probably produces a dimeric circle which is resolved through recombination to produce a plasmid containing an inverted repeat structure of the termini (224). When circular plasmids containing such a head-to-head structure (isolated from concatenated replicative structures) are placed in virus-infected cells, the ends are replicatively resolved, converting the plasmid progeny to a linear structure with vaccinia termini and plasmid sequence in between (225). The ability of the concatenated end joint to resolve a circular plasmid to a linear structure is dependent upon the presence of at least 252 base pairs of concatamer joint (about 125 nucleotides from each terminus). Plasmids with 132 base pairs of concatamer joint are unable to resolve into linear structures. The role, then, of the terminal 70-bp repeat structures (which start at nucleotide 87 from the terminus) in replication is unclear (225).

E. The Direct Approach: Protein Primers

Perhaps the most direct method for supplying the requirement for a 3'OH primer is to utilize a nucleotide linked to a protein which recognizes the 3'-OH terminus of the template strand. Adenovirus uses such a system (reviewed in chapter 20 of this volume and ref. 226-229), as does the bacteriophage Φ29 (230).

Adenovirus

Adenovirus is packaged as a linear, double-stranded DNA molecule with a terminal protein covalently linked to the 5' deoxycytidine of the terminal inverted repeat ends through a phosphodiester bond to serine (231). The terminal protein (TP) is a proteolytic product of the precursor terminal protein (pTP) seen at the 5' end of newly synthesized strands. Proteolytic processing occurs late in infection (232) and uses a viral-encoded protease. The ends of adenovirus appear to be circular, but the linkage is usually not covalent. Rather, the TP at the 5' ends interact to circularize adenovirus. However, true covalently-closed circles do occur after infection (233, 234) and these head-to-tail circles are infectious (234). The role of these circles in replication is unclear. Their possible role in integration will be discussed in Section 2 F. of this review.

The initiation reaction adds dCTP to pTP in the presence of either end of the ds viral DNA and viral polymerase. The viral template DNA can either have TP (or pTP) on the to-be-displaced parental strand or can have the TP completely removed. Incomplete removal, however, leaving peptides on the parental strand inhibits initiation (235). The reaction also requires Mg^{++} and ATP (GTP or dATP can substitute) (236). The ability of deproteinized, and, hence, linear, double-stranded adenovirus DNA to support initiation, albeit poorly, indicates that circularization

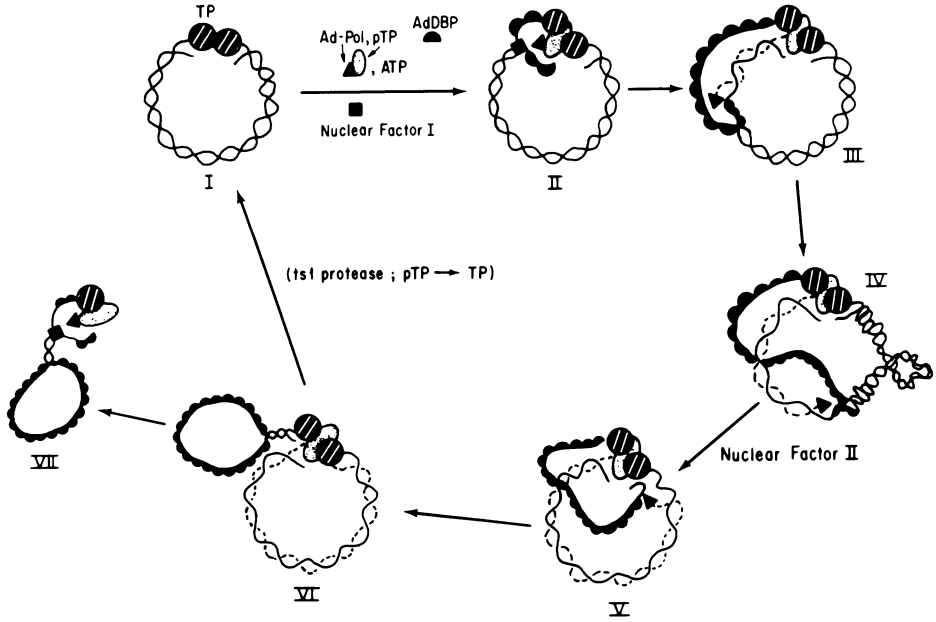

Figure 11. Adenovirus replication. See text for discussion. Reprinted with permission (reference 228).

is not crucial for replication initiation. However, the normal replicative form of the virus *in vivo* is probably circular (see Fig. 11).

Utilizing linearized plasmid constructs with variable amounts and organization of viral terminal sequences, it has been seen that the initiation event can tolerate two (but not three) additional G/C pairs (235, 237), can tolerate removal of up to eight terminal base pairs (238) but cannot tolerate termini which have less than 19 base pairs of viral sequence (235). Site-directed mutagenesis to the conserved region between positions 9-17 (239) destroyed all template activity. Although this region is an important recognition sequence for initiation and replication, it is probably not sufficient, since adenovirus AdF1 DNA/TP is inactive as a template in an *in vitro* system with proteins from Ad2 infected cells, even though AdF1 and Ad2 share identical terminal sequences through base 17 (237). The converse is also true, Ad2 DNA/TP is inactive in an in vitro system from AdF1 infected cells. This may reflect a strain-specific interaction between the TP on parental strands and new pTP/polymerase.

In addition to the pTP, viral replication utilizes a viral-encoded polymerase which can be found as the pTP-polymerase complex which binds to nucleotides 9-22 at the viral termini (240). Both pTP and polymerase are necessary to synthesize pTP-dCMP, and the viral polymerase cannot be replaced by other polymerases (241). This region slightly overlaps a region (nucleotides 17-48) protected from nuclease digestion by host-derived nuclear factor I (242), which, in the presence of ATP, stimulates initiation by the pTP-polymerase complex. A viral-

encoded nonspecific single-stranded DNA-binding protein (DBP), although not necessary for the initiation phase, does greatly stimulate initiation when host nuclear factor I is present (243). In the absence of host nuclear factor I, DBP inhibits initiation (243). The host nuclear factor I does not affect the binding of pTP-polymerase complex to single-stranded viral DNA, such as a displaced parental strand, although this reaction is inhibited by viral DBP (244). These data suggest that nuclear factor I and DBP may interact in opening up a region of viral termini which can be recognized by pTP-polymerase (228). Significant elongation of the newly-initiated strand does, however, require viral DBP, and this function cannot be replaced by DNA binding protein from *E. coli* (228).

In addition to DBP, full-length elongation of viral DNA, which proceeds only in the direction of fork movement with displacement of parental strand, requires a second host factor (nuclear factor II) which has topoisomerase activity (228, 242). In the absence of nuclear factor II, replication stops at around 10,000 bp. This factor can be replaced by eucaryotic topoisomerase I. The activity of factor II requires the presence of factor I, as does eucaryotic topoisomerase I (245). *E. coli* topoisomerase will not replace either eucaryotic topoisomerase (245) in permiting full-length adenovirus replication.

Once initiation begins, apparently randomly, at one of the ends of the virus, it appears that this end is preferentially re-initiated, and molecules which have initiated at both ends are only rarely seen. This process, then, leads to displacement of a single-stranded parental DNA. Synthesis of the complementary strand is subsequently initiated on the displaced strand in a manner reminiscent of reovirus replication (see section 1 C.). Whether the normal structure of the single-stranded parental strand, upon separation from the replication complex, involves hybridization of the terminal inverted repeats and, hence, formation of a duplex terminus strictly analogous to the termini of duplex virus, is not known.

A similar, though slightly different, process is involved in Φ29 synthesis (230, 246, 247). The main difference is that the terminal protein on the parental strand is absolutely required for initiation (247) of DNA synthesis by a new terminal protein to which dAMP will be added — i.e: protein-protein interaction of the two terminal proteins is required for initiation. Full-length virus can be made *in vitro* with only the terminal protein and viral polymerase, although host factors and other viral factors may stimulate activity (246).

F. Viral Integration and Transposition

A number of DNA viruses in bacteria (e.g., mu, lambda) and in animals (including some papovaviruses, adeno-associated virus, adenovirus, the DNA forms of hepatitis B and retroviruses, and possibly herpesvirus) are capable of integrating into host DNA. In some cases, most notably in bacteriophage, retroviruses, and adeno-associated viruses (AAV), integration into host DNA can play a role in the viral life cycle. For some of these viruses — e.g., retroviruses and bacteriophage mu — integration is crucial to their productive infection.

In other bacteriophage and in AAV, integration represents an alternate life style to productive infection. Typically, once integrated, the virus is quiescent, ex-

pressing only a few, if any, viral functions, and replicating as part of the host chromosome. In this integrated structure, the virus can often escape host mechanisms which are designed to protect the host from viral infections. The virus remains in a cryptic state until an environmental signal rescues it and productive infection begins again. In some of the bacteriophage, the shut-down of most viral functions by integrated virus also protects the host from lytic infection by the same or related viruses. In the case of herpesviruses and hepatitis B viruses, viral latency occurs, as does at least partial integration. However, it is unclear whether there is any relationship between latency and integration.

In many other animal DNA viruses which integrate into host DNA (adenoviruses, polyoma, SV40, and, possibly, hepatitis B and herpesviruses), integration is probably a replicative dead-end, although integration is often (but not always — e.g., in papilloma viruses and, possibly, herpesviruses) required for oncogenicity. Typically integration of these viruses is a relatively rare event, occurs in hosts which are semipermissive or nonpermissive for productive infection, and often results (either initially or over time) in rearrangement or deletion of viral information.

In this section we will discuss viruses in terms of the specificity of the process of integration and excision. However, one should remember that integration is often a rare event, and little is known about it except from those cell lines transformed by the process, often long after secondary rearrangements may have occurred.

Site-specific integration

In the first class of integration events we will examine, integration occurs at specific homologous sequences in both the virus and host. The best characterized system of this type, of course, is bacteriophage lambda (see ref. 159, 160, 161, 248). Integration and excison are *conservative* events; that is, replication is *not* involved. The integration/excision events require viral and host functions.

In order to integrate, λ DNA must be in a supercoiled circular form. Integration requires the product of the *int* viral gene, which exhibits type I topoisomerase activity, and two host-encoded proteins (Him A and Him B).

The *int* product binds to several sites near and in the phage attachment site, and produces a staggered cleavage 7 base pairs apart, in the 15-bp core homologous region in both virus and host, with consequent sealing leading to physical integration.

Excision also requires the *int* topoisomerase, but, in addition, requires the product of the viral *xis* gene, probably to provide a directionality (excision *vs.* integration) to the *int* reaction. Although such a process, with specificity in both host and viral sequences, is common in bacteriophage, it is unknown in animal viruses.

Integration at non-specific sites

In the second type of integration event, integration does not involve specific sequences on either host or virus. The best examples of this type of integration are polyoma and SV40.

Integration sometimes results in (or from) extensive deletion of chromosomal DNA (249). The sites of integration show no extensive homology to viral sequences and there are no repeated flanking host sequences. There is no particular integration site on the virus. The integration process appears to be entirely host-directed and can probably utilize any input DNA. Occurring in nonpermissive hosts, this non-specific integration is a rare event (250) which is probably not normally a part of viral function. Typically, in such illegitimate recombination, some viral functions continue. In the case of polyoma and SV40, the continued synthesis of T-ag is a necessary (but not sufficient condition) for their ability to transform cells. Since many of the integrated viral sequences are present as multimers or partial multimers in a head-to-tail arrangement, a model of integration involving linear concatamers generated by a (probably aberrant — since it occurs in nonpermissive cells) rolling circle mechanism has been suggested (251). The presence of multimers probably is necessary for the regeneration of complete viral circles via homologous recombination.

Excision of viral DNA and production of circular virus appears to involve the replication of integrated viral sequences independently of host DNA (requiring a functional viral origin and T-ag sequence) in combination with homologous recombination (252,253). This process not only produces viral circles, but can eventually lead to the spontaneous elimination of integrated virus from cell lines.

Integration at preferred sites

The third type of viral integration in animal viruses involves no specific sites in host DNA, but there are preferred sites by which the virus integrates — namely sequences at or near one or both of the ends of a linear viral form. Examples are adenovirus, AAV, and hepadna viruses. Of these, the most interesting, in terms of replication, is AAV, which is the only one which can normally be rescued.

AAV provides an interesting model which may be suitable for understanding the integration of adenovirus and hepadna virus. Interestingly, the autonomously replicating parvoviruses (ARPV) have not been found to be integrated perhaps because of their limited host ranges. However, in the case of a plasmid construct containing an ARPV, the virus can be rescued if the 5' hairpin region is present (254), indicating that, in this case, rescue may be a distinct process from integration. Integration of AAV occurs naturally, although rarely, in the absence of helper virus (i.e., under nonpermissive conditions). Integration appears to preferentially involve the terminal inverted repeats (255). Often, a head-to-tail multimer (within the limit of restriction enzyme analysis) is seen to be integrated, although multimers are not necessary for rescue (186, 197). Rescue of integrated AAV can be initiated by infection with adenovirus which, of course, is necessary for productive infection. Rescue of virus from plasmid constructs with specific terminal deletions indicate that rescue involves a specialized form of replication and requires that at least one terminal palindrome remain either intact or be repairable by replication (see section 2 D.) (197, 256). It probably also requires sufficient sequence of the other palindrome so that repair synthesis of a panhandle structure would regenerate a complete end.

Adenovirus integration

Adenovirus integration into nonpermissive host cells has been recently reviewed (257). There is no evidence that adenovirus integration occurs at a specific site in host DNA. Although there are some short homologies between viral and cellular sequences which may play a role in the integration process, these do not exceed the statistically expected values. Like SV40 and polyoma, there is no duplication of cellular nucleotides at the junction between viral and host sequence (thus ruling out a transposition-like event), and, in some cases, no deletions of cellular nucleotides. Integration typically (but not always) occurs near, but not at, the termini of viral DNA. Deletions of 2, 8, 10, 45, 64, and 174 nucleotides of viral terminal sequences have been observed.

As we saw in Section 2 E. small deletions (less than 13 nucleotides) at the head-tail junction of the circular form of adenovirus do not prevent infectivity (234), and small deletions (less than 8 nucleotides) in the terminus of adenovirus do not prevent initiation of replication (238).

However, in contrast to the circular form of adenovirus in which the *viral* ends are joined (234), plasmids in which the viral ends are linked to plasmid sequences are not infectious unless the ends are cleaved (258) and free. This implies that integrated adenovirus would not normally be rescueable. The presence of nearly complete adenovirus integrates which end near the termini (257) and the occasional appearance of integrates with linked termini (259) indicate that circular forms may sometimes play a role in integration. Unlike SV40 and polyoma, multiple insertions are usually not true tandem repeats, but are separated by other (host) sequences (257). Secondary re-arrangements of adenovirus integrates are common, as is eventual loss of viral sequences.

Integration of Hepatitis B virus DNA

When integrated hepatitis B virus in hepatocellular carcinomas are examined, one often sees extensive rearrangement of both viral (260, 261) and host sequences (262). But in several cell lines without extensive rearrangement, one viral/cell junction is near the site of initiation of the reverse transcriptase while the other junction is found at various sites in the viral genome (263). In some cases, flanking host sequences are duplicated as direct repeats of various lengths; in other cases they are not. The mechanism of integration may be similar to that suggested as the way small nuclear RNA pseudogenes can be generated from cDNA copies via integration at a staggered cut in host DNA (264).

Hepatitis B at the stage of reverse transcription is a single-stranded DNA molecule, as is AAV, and as is, at least in some stages of the replicative cycle, adenovirus. It may be that this is a common feature of the integration of these viruses into host chromosomes, perhaps by a process of patch homology recognition to sites in host DNA which are temporarily single-stranded themselves.

In the case of adenovirus, it may be that single-stranded ends of a double-stranded virus rather than a true single-stranded replicative intermediate induces integration. This may account, because replication would be unnecessary, for the

failure to find terminal direct repeats of host sequences at the junctions of adenovirus integrates (257) as is also the case with the double-stranded polyoma or SV40 integrates. Normally, only in the case of AAV does the integrated form play a role in the replication of virus. This may be because minor deletions at the viral termini in AAV can be regenerated during its replication and mechanisms for specific DNA cleavage are part of its replication mechanism.

Transposition and integration

The last type of viral integration involves transposition, whereby a specific viral transposase makes a staggered cut in host DNA, ligates the precise ends of the virus to the single-stranded extensions, and introduces viral DNA, either conservatively or by replication into the site which now has flanking host direct repeats. Retroviruses (see Section 3 of this review and chapter 16) are the animal viruses which transpose. In this section, however, we will examine bacteriophage mu.

Bacteriophage Mu

Bacteriophage mu replicates exclusively by a transposition-directed process, making this virus unique and also allowing it to be studied as a model transposable element (265). The ability to analyze mu-dependent replication in defined *in vitro* systems (265, 266) has permitted the trapping and analysis of the transposition intermediate (Fig. 12) (267). Transposition to the intermediate stage requires that both mu ends be present and in the proper orientation in a supercoiled structure

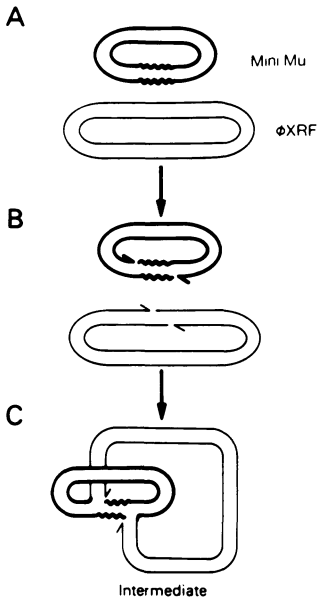

Figure 12. Formation of phage mu replicative intermediate. A. Plasmid with mini Mu transposon and ΦX174 RF as recipient. B. Introduction of staggered (5bp) cut in recipient DNA and cuts at termini of mu transposon. C. Formation of integration intermediate. Completion of transposition can either be replicative to yield a cointegrate or involve cleavage and loss of the non-mu donor DNA. Reprinted with permission (reference 268).

(268). Unlike most transposons, the ends of mu are not identical inverted repeats, but do contain sequences recognized by the viral transposase (269), the gene A product. The transpose specifically makes a staggered cut in the acceptor DNA, nicks the precise ends of the phage, and ligates the 3' ends of the phage to the 5' extensions of the acceptor DNA. This forms the *transposition intermediate.*

Once the transposition intermediate is formed, either co-integrate formation or simple insertion can proceed using only host enzymes (268). Co-integrates form by the initiation of DNA replication starting at the 3' end of the host DNA which is at the viral left-most end of the transposition intermediate; simple inserts require degradation of the DNA which originally flanked the transposon, followed by gap repair (270). In either case a 5-bp direct flanking repeat of host DNA is produced indicating a 5-nucleotide staggered cut in the case of the mu phage. To produce the transposition intermediate, one needs, for high efficiency, ATP, Mg^{++}, Mu A (the transposase) protein, Mu B protein (Mu B protein binds to DNA non-specifically (271) and is not absolutely required for mu transposition (272) but acts as a strong enhancer of transposition), and a host protein recently identified as HU protein (a nonspecific DNA-binding protein) (273). In addition, the mu phage must be supercoiled although the acceptor DNA can be supercoiled, relaxed, or linear (273). Since mu phage is packaged as a linear molecule with the viral information imbedded in random flanking host DNA, the phage should not be in the form of covalently closed circles. However, a circular, and thus potentially supercoiled form of mu in which the ends are held together by a protein bridge has been observed after infection (274).

3. VIRUSES THAT USE REVERSE TRANSCRIPTASE

The last group of viruses we will examine are unique in that genomic information switches between RNA and DNA, aided by a unique polymerase able to make a DNA copy of a pregenomic mRNA. Included are the retroviruses, the hepadna viruses, and cauliflower mosaic virus.

In the case of retroviruses, it is the pregenomic mRNA which is packaged in the virion. When this RNA virion enters the cell, it is converted to a double-stranded DNA copy with duplicated information at the termini (the long terminal direct repeats — the LTRs). These direct LTR repeats also include a small inverted repeat at their ends. Fig. 13 shows the proposed mechanism of the conversion of virion RNA of retroviruses to a double-stranded DNA, a process which is extensively discussed in chapter 16 of this volume. The reader is also referred to recent reviews (275, 276).

Virion reverse transcriptase activity requires a primer for initiation. In the case of retroviruses, the primer for (-) strand DNA synthesis is provided by the 3' end of a host tRNA which is complementary to a region of viral RNA. Synthesis proceeds to the 5' end of the virion RNA, which is terminally repeated at the 3' end (Fig. 13).

RNase H activity (associated with reverse transcriptase) removes 5' RNA sequences hybridized to newly-synthesized DNA (Fig. 13. 3), leaving a single-stranded DNA which is complementary to the direct repeat R region RNA at the 3'

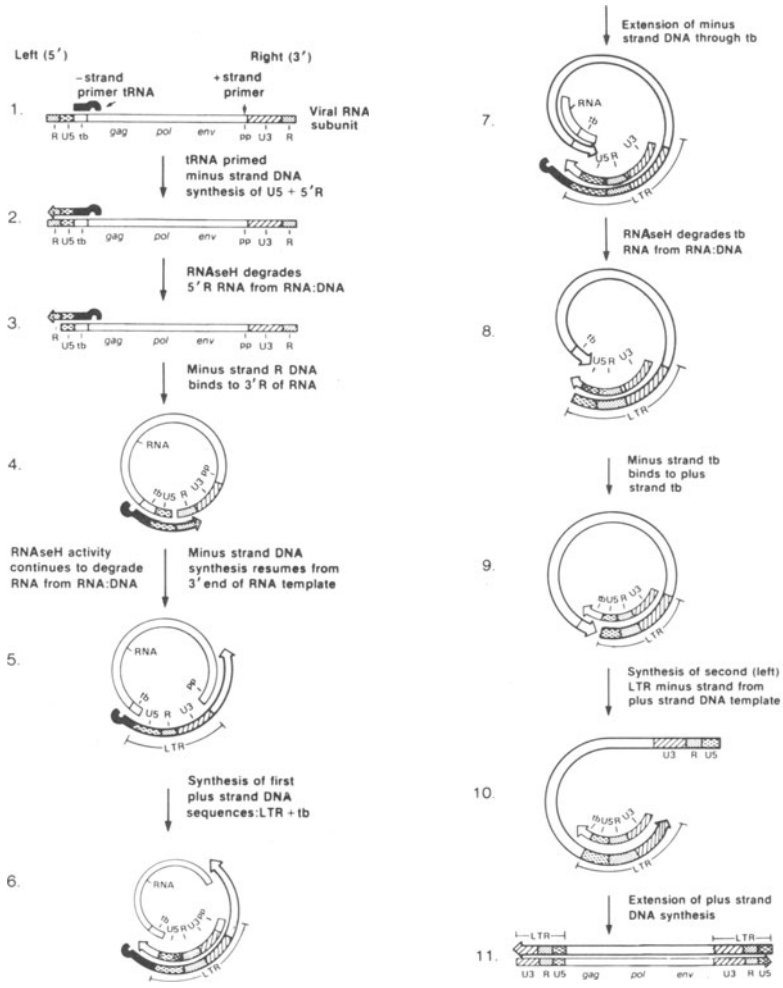

Figure 13. Replication of retrovirus. See text for description. Reprinted with permission (reference 276).

end of the same molecule (or of the other virion RNA packaged with it, since the genomic RNA of retroviruses is packaged as *dimers*). Hybridization of the free single-stranded DNA to the direct repeat produces a circular (or concatameric if to the other virion RNA) molecule (Fig. 13. 4), and transcription of (-) strand and RNase H activity continue (Fig. 13. 5). A short sequence of template RNA just past the LTR sequence (Fig. 13. 6), generated by its resistance to RNase H activity, is the primer for (+) DNA strand synthesis (277-280). Plus strand synthesis (using the primer) continues through at least 18 nucleotides of the tRNA primer of the (-) strand before stopping (281).

Minus strand synthesis, meanwhile, proceeds through to the 5' end of the RNA template (Fig 13.7), which, because of RNase H activity, has been trimmed back to the sequence complementary to tRNA (Fig. 13.8). This remaining viral RNA sequence was not removed by RNase H because it involved RNA:RNA pairing. Rather, the tRNA was displaced by (+) strand synthesis. This tRNA-binding region, where (-) DNA strand synthesis stops, is, after removal of RNA template, complementary to the 18 nucleotides or so extension of the (+) DNA strand, which is also single-stranded DNA because of RNase H removal of the displaced tRNA primer (Fig. 13. 9).

The (-) strand 3' end, then, will hybridize to the complementary (+) strand 3' end. The molecule is now a mostly single-stranded (-) DNA circle with a nick whose ends are held together by an overlapping (+) complementary DNA strand initiated at the LTR junction (Fig. 13. 9). Displacement synthesis from the (-) 3' end and continued synthesis at the (+) 3' end (Fig. 13. 10) will now produce a linear DNA with a direct repeat LTR at each end (Fig. 13. 11). This structure acts like a transposable element and will integrate into host DNA.

For retroviruses, integration is an important element in the replicative cycle. Integration results in the deletion of the 2 terminal nucleotides from the linear double-stranded DNA structure. Integration does not appear to depend upon host sequence (282), requires the terminal inverted repeats (283), and results in a 4-6 nucleotides (depending upon the virus) flanking direct repeat of host DNA. In addition to the endonuclease function of the *pol* viral gene, which recognizes specific sequences in the viral terminal inverted repeats (284), cellular functions are also required (285).

The integrated virus then initiates transcription at the left end of the R sequences in the left-hand LTR, utilizing host enzymes. There is a poly(A) termination signal about 20 nucleotides upstream from the right-hand end of the R sequences, which, however, is not utilized when first encountered after initiation of the transcript. It is utilized, however, when encountered at the right-hand end of the virus (that is, when full-length viral transcript is made) and poly(A) is added to sequences downstream of the R sequences producing a terminal redundancy of R sequences. This transcript is both the viral primary mRNA, which can be processed by splicing, and the virion RNA genome, which is encapsidated.

Hepadnaviruses

Hepadnaviruses (Hepatitis B) and cauliflower mosaic viruses, although packaged as DNA virions, share features which make them appear to be more closely related to the retroviruses than to the other DNA viruses (286, 287). In particular, all three groups utilize a reverse-transcription step in the production of DNA virus (288, 289). In turn, the DNA virus is the source of the RNA transcript used as template during the reverse transcription.

The analysis of hepatitis B viral replication has been hampered by the extremely hepatotropic nature of the virus. Infection of hepatocytes requires a cellular function which is quickly lost (within 7 days) from hepatocytes in culture (290), so most analysis must be done with *in vivo* infections rather than the more

manipulable tissue culture infections. Although hepatocytes are the primary focus of infection, hepadnaviruses can infect other tissues *in vivo* including a small fraction of peripheral blood lymphocytes where they reside primarily in episomal (latent ?) form (291). In hepatocytes integration of virus is relatively common — although monomeric circles are much more common. The differential importance of integrated and non-integrated forms of hepadnaviruses is not clear.

Hepatitis B virus is packaged as a partially double-stranded DNA circle (for a detailed review, see chapted 17). The (-) strand is full-length and contains a 5'-terminal covalently-linked protein at a specific nick site. The (+) strand overlaps this nick and has heterogenous 3' ends. When the partially double-stranded DNA virion enters an hepatocyte, it is converted to a complete duplex supercoiled circular minichromosome. Multimeric circles and integrated virus are also produced.

The covalently-linked protein is assumed to act as a primer for reverse transcription of the pregenomic RNA (see Fig. 14). The pregenomic RNA contains a terminal redundancy of 270 nucleotides produced by synthesis of a more than genome length RNA, a process which requires that a polyadenylation signal in the double-stranded DNA circle be ignored (295) when first encountered. The initiation site for pregenomic RNA synthesis is just upstream of one of two direct repeat sequences (292) found near or at the ends of the cohesive overlap region — the region between the 5' end of the (-) DNA strand and the 5' end of the (+) DNA strand of the packaged virion.

Elongation of the (-) DNA strand off pregenomic RNA appears to occur concommitantly with degredation of the RNA template by RNase H activity, so that (-) strand DNA appears to be single-stranded rather than templated to RNA (288, 293). The complementary (+) strand DNA is initiated on single-stranded (-) DNA with a capped oligoribonucleotide of 18-19 bases which differs at its 5'-most 6 nucleotides from the sequence to which it is bound, one of the 12-13 nucleotide direct repeat sequences. This primer, however, is identical to the sequence at the 5' end of the (+) pregenomic RNA used as a template for reverse transcriptase (see Fig. 14). The suggestion is that, like retroviruses, this oligoribonucleotide primer is "borrowed" from pregenomic RNA (or another RNA which starts at this site) (294). The newly synthesized (+) DNA, which, at it's 3' end, is complementary to the sequence at the 5' end of the (-) DNA, then "jumps" to hybridize to that end. In this process, the terminal protein and some 7-8 nt are displaced. Thus the (-) DNA "circle" has a small 7-8 nucleotide single-stranded 5 "wisker" with terminal protein attached. At this point, replication of (+) strand DNA continues on the now "circularized" (-) DNA strand. Typically, (+) DNA replication terminates before the strand is completed, leaving a partial (+) DNA strand with heterogeneous 3'-termini (296).

Cauliflower Mosaic Virus

Cauliflower mosaic virus replication appears to be similar (289, see Fig. 15). The encapsidated virion is a circular double-stranded molecule with three interruptions and all have small single-stranded 5' duplications which, upon entry into host, are trimmed back, allowing the virus to become a covalently closed double-

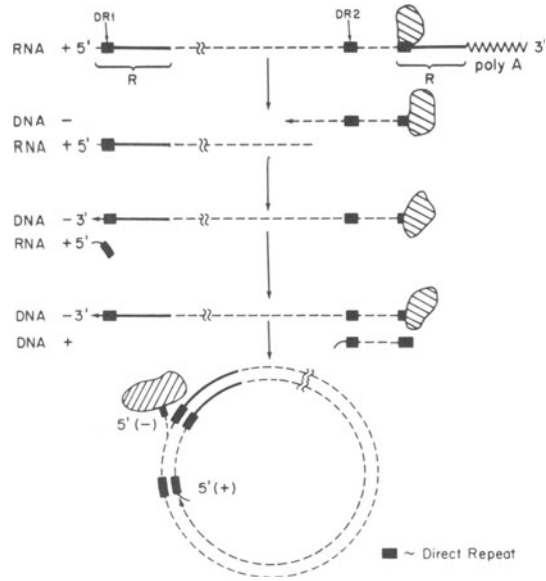

Figure 14. Replication of hepadna virus. See text for description. Reprinted with permission (reference 294).

stranded DNA circle. The virion is then found in the nucleus as a supercoiled minichromosome (297) where, along with subgenomic mRNAs, it produces a pregenomic RNA with a terminal redundancy of 180 nucleotides (298). Again a transcriptional termination signal must be ignored upon first being encountered. At this point, it is proposed that reverse transcription (using a viral polymerase (299) which has homology to retroviral reverse transcriptase (287) and exhibits reverse-transcriptase activity *in vitro*) produces (−) strand DNA. There is some evidence that (−) strand production occurs in virioplasms in an asymmetric fashion (300) and it has been suggested that, like retroviruses, the primer is a host tRNA (289). After synthesis of (−) DNA to the 5' end of the pregenome RNA, a switch to the terminally redundant 3' end of pregenome RNA is suggested with continued replication to produce a full-length (−) strand. The (−) DNA remains circular due, possibly, to hybridization to part of the RNA template not removed by RNase H activity. It is slightly redundant because a small amount of displacement synthesis occurs when the polymerase encounters the double-stranded DNA/RNA hybrid near the site of initiation on the circular RNA. Synthesis of (+) strand initiates at two sites, in regions which have short stretches of very high G content RNA, which may be RNase H resistant. It is suggested that these RNAs act as primers for (+) DNA strand synthesis. One of these (+) strands must "jump" across the slightly redundant gap in the (−) strand and continue synthesis until the 5'-end of another (+) strand is met. In all cases, the polymerase appears to continue, via displacement of the 5' end, through any remaining 5' RNA primer and 5,

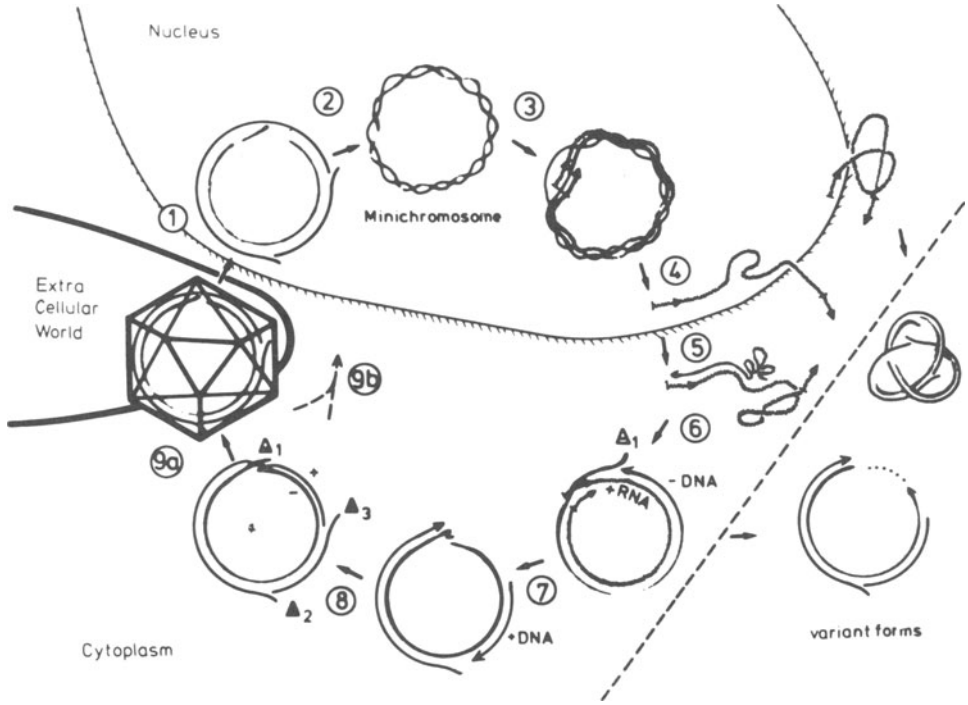

Figure 15. Replication of cauliflower mosaic virus. See text for description. Reprinted with permission (reference 289).

15, and 18 deoxynucleotides for nicks 1, 2 and 3 respectively, thereby producing a small single-stranded terminal 5' redundant region at each nick. The length of these single-stranded "wiskers" varies slightly.

Two remarkable features emerge from these data:

i) replication of all three viruses requires redundant regions in a pregenomic RNA which is produced by ignoring a transcriptional termination signal upon first encounter. This allows switching of DNA/RNA hybrids from one site on the pregenomic RNA to another when the RNA template is removed by RNase H activity; and

ii) displacement synthesis can occur when polymerase encounters a duplex region, again producing small redundancies useful for circularization or generating terminal duplications, as in retrovirus LTRs.

4. CONCLUDING REMARKS

Although there is no one mechanism of viral replication, one is struck by the extent to which RNA viruses utilize host transcriptional and translational proteins to perform the task of assisting the viral replicase recognize the 3' ends of the

template. The presence of structural features which are tRNA-like at the ends of a number of RNA viruses are a further manifestation of the use of host translation mechanisms. The use of these proteins probably reflects the requirement, shared by RNA viruses (but not some viral satellites and viroids), that replication start at the 3'-OH end of a linear template molecule. Although RNA viruses can use various methods for initiation of replication, including primers such as protein primers and host mRNA sequences (although these latter are used only in transcriptional products), primers are not a necessity for all RNA viruses. Moreover, some RNA viruses take advantage of the ability to add ribonucleotides in a non-template-directed manner (adding terminal CCA-3' via tRNA-associated enzymes; 5-methylguanosine cap; poly (A) 3'-tail) to either complete a full-length transcript, to help in positioning transcription/replication proteins, or to distinguish between transcriptive or replicative molecules.

RNA viral replication always involves displacement synthesis (sometimes only after RF formation), a feature probably required by the lack of RNA unwinding enzymes. The ability to recognize and to initiate RNA synthesis directly at an end is probably the reason why no RNA virus uses a scheme similar to the parvoviruses, a scheme which otherwise is compatible with RNA replication.

DNA viruses, in contrast, require a 3'-OH primer for DNA polymerase activity. For most DNA viruses, the primer is supplied by an RNA oligonucleotide (which is subsequently replaced by DNA) in a manner similar to that observed in host DNA replication. The requirement for a primer can also be satisfied by 3'-OH sites in double-stranded DNA nicked at specific loci. Such 3'-OH DNA primers can either be generated in viral hairpins (e.g. poxvirus, parvovirus), at a junction with flanking host DNA (e.g. phage mu), in double-stranded circles, permitting concatamer formation (e.g: ΦX174), or in invasive recombination-like processes (e.g. T4). Finally, the primer requirement can be satisfied by a specific priming protein (e.g. adenovirus).

Because DNA unwinding enzymes are available, DNA viral replication can (but need not) initiate internally and proceed in a semi-conservative fashion on *both* strands (i.e. the displaced or lagging strand is also replicated) and in *both* directions from the initiation site. Internal initiation and the need for a 3'-OH primer requires either that there are no ends in the virus (i.e. the molecule is circular) or that other mechanisms are in place for resolving ends. Retrograde DNA synthesis on the lagging strand requires multiple initiations, primer removal, and ligation of DNA. This is an enzymatically more complex process than simple displacement synthesis and, often, host enzymes are utilized. The absence of a double-stranded RNA nicking/sealing activity similar to DNA ligase activity is probably one reason why RNA viruses only initiate at ends and by displacement of strands.

The viruses that utilize both RNA and DNA genomes, using the unique reverse-transcriptase activity to switch between them, all require production of RNA genomes (pregenomes) which have terminal redundancies. Although the RNA form is not circular, the DNA form becomes circular by hybridization of single-stranded DNA ends which have had their RNA templates removed by RNase H activity. The reverse transcriptase, like all DNA polymerases, requires a primer

which can be supplied by either a host tRNA (retroviruses and cauliflower mosaic virus) or by a protein (hepadna virus). It can also produce strand displacement synthesis, a feature which can generate ssDNA redundancies necessary for hybridization or to generate a linear DNA with terminal redundancies.

The enzymology of viral replication appears to require enzymes which are either host enzymes or are modifications of host-like enzymatic activity. There are no truly unique enzymatic activities (e.g. a 3' to 5' polymerase) encoded by viruses. Although priming proteins do not appear, as such, in host cells, proteins with covalently-linked nucleotides are not unusual and some are also oligonucleotide-binding proteins. In many cases, the covalent-linkage is only transient and involved in energy transfer. However, no great leap is required to transform such a protein to a priming protein.

Thus, despite the wide variability in mechanisms of viral replication, one observes limitations in the range of possible mechanisms which are related to the nature of the genome (RNA or DNA) and the ways that cells deal with these molecules in their normal functioning. This, of course, is what one would expect in a parasitic entity which evolved from a free-living progenitor (although not necessarily evolved by loss of function — the acquisition of mobile, infectious status by a cell component is also possible), and which has co-evolved with its host by ex-ploiting its normal functions.

5. REFERENCES

1) D.C. Reanny (1982) Ann. Rev. Microbiol. **36**, 47-73.

2) Matthews, R.E.F. (1983) In *A Critical Appraisal of Viral Taxonomy* Matthews, R.E.F., ed. CRC Press, Boca Raton, 220-245.

3) Silverstein, S.C., Christmas, J.K., & Acs, G. (1976) Ann. Rev. Biochem) 45, 375-408.

4) Ahlquist, P., Dasgupta, R., & Kaesberg, P. (1981) Cell **23**, 183-189.

5) Goelet, P., Lomonossoff, G.P., Butler, P.J.G., Akam, M.E., Gait, M.J., & Karn, J. (1982) Proc. Natl. Acad. Sci. USA **79**, 5818-5822.

6) Ahlquist, P., Dasgupta, R., & Kaesberg, P. (1984) J. Mol. Biol. 172, 369-383.

7) Blumenthal, T. (1979) Ann. Rev. Biochem. **48**, 525-548.

8) Bastin, M. & Hall, T.C. (1976) J. Virol. **20**, 117-122.

9) Bishop, D.H.L., Gay, M.E., & Matsuoko, Y. (1983) Nucleic Acids Res) **11**, 6409-6418.

10) Patterson, J.L. & Kolakofsky, D. (1984) J. Virol. **49**, 680-685.

11) Krug, R.M., Broni, B.B., & Bouloy, M. (1979) Cell **18**, 329-334.

12) Plotch, S.J., Bouloy, M., Ulmanen, I., & Krug, R.M. (1981) Cell 23, 847-858.

13) Winter, G., Fields, S., Gait, M.J., & Brownlee, G.G. (1981) Nucleic Acids Res. **9**, 237-245.

14) Lamb, R.A. & Choppin, P.W. (1983) Ann. Rev. Biochem. **52**, 467-506.

15) Francki, R.I.B. (1985) Ann. Rev. Microbiol. **39**, 151-174.

16) Diener, T.O. (1979) Science **205**, 859-866.

17) Rackwitz, J.W., Davies, C., Hatta, T., Gould, A.R., & Francki, R.I.B) (1981) Virology **108**, 111-122.

18) Gerlach, W.L., Buzayan, J.M., Schneider, I.R., & Bruening, G. (1986) Virology **151**, 172-185.

19) Symons, R.J., Haseloff, J., Visvader, J.E., Keese, P., Murphy, P.J., Gordon, K.H.J., & Bruening, G. (1985) In *Subviral Pathogens of Plants and Animals: Viroids and Prions* Maramorosch, K. & McKelvey, J.J., eds) Academic Press, New York, 235-263.

20) Branch, A.D., Willis, K.K., Davatelis, G., & Robertson, H. (1985) In: *Subviral Pathogens of Plants and Animals: Viroids and Prions*, Maramorosch, K. & McKelvey, J.J., eds. Academic Press, New York, 201-234.

21) Haenni, A.-L., Joshi, S., & Chapeville, F. (1982) Prog. Nucl. Acid Res) Mol. Biol. **27**, 85-104.

22) Inouye, H., Pollack, Y., & Petre, J. (1974) Eur. J. Biochem. **45**, 109-117.

23) Blumenthal, T., Landers, T.A., & Weber, K. (1972) Proc. Natl. Acad. Sci) USA **69**, 1313-1317.

24) Franze de Fernandez, M.T., Eoyang, L., & August, J.T. (1968) Nature (London) **219**, 588-590.

25) Rensing, U. & August, J.T. (1969) Nature (London) **224**, 853-856.

26) Weber, H. & Weissman, C. (1970) J. Mol. Biol. **51**, 215-224.

27) Prochiantz, A., Benicourt, C., Carre, D., & Haenni, A.L. (1975) Eur. J) Biochem. **52**, 17-23.

28) Bujarski, J.J., Dreher, T.W., & Hall, T.C. (1985) Proc. Natl. Acad. Sci) USA **82**, 5636-5640.

29) Dreher, T.W., Bujarski, J.J., & Hall, T.C. (1984) Nature (London) **311**, 171-175.

30) Ahlquist, P., French, R., Janda, M., & Loesch-Fries, L.S. (1984) Proc) Natl. Acad. Sci. USA **81**, 7066-7070.

31) Miller, W.A., Dreher, T.W., & Hall, T.C. (1985) Nature (London) **313**, 68-70.

32) Rueckert, R.R. (1985) In *Virology* Fields, B.N., ed. Raven Press, New York, 705-738.

33) Perez-Bercoff, R. (1978) In *Molecular Biology of Picornaviruses* Perez- Bercoff, R., ed. Plenum Press, New York, 319-330.

34) Nomoto, A., Lee, Y.J., & Wimmer, E. (1976) Proc. Natl. Acad. Sci. USA **73**, 375-380.

35) Flanegan, J.B., Petterson, R.F., Ambros, V., Hewlett, M.J., & Baltimore, D. (1977) Proc. Natl. Acad. Sci. USA **74**, 961-965.

36) Golini, F., Semeler, B.L., Dorner, A.J., & Wimmer, E. (1980) Nature (London) **287**, 600-603.

37) Sanger, D.V. (1979) J. Gen. Virol. **45**, 1-11.

38) Ambrose, V. & Baltimore, D. (1980) J. Biol. Chem. **255**, 6739-6744.

39) Spector, D.H. & Baltimore, D. (1974) Proc. Natl. Acad. Sci. USA **71**, 2983-2987.

40) Van Dyke, T.A. & Flanegan, J.B. (1980) J. Virol. **35**, 732-740.

41) Andrews, N.C., Levin, D.H., & Baltimore, D. (1985) J. Biol. Chem) **260**, 7628-7635.

42) Andrews, N.C. & Baltimore, D. (1986) Proc. Natl. Acad. Sci. USA **83**, 221-225.

43) Andrews, N.C. & Baltimore, D. (1986) J. Virol. **58**, 212-215.

44) Young, D.C., Dunn, B.N., Tobin, G.J., & Flanegan, J.B. (1986) J. Virol) **58**, 715-723.

45) Hey, T.D., Richards, O.C., & Ehrenfeld, E. (1986) J. Virol. **58**, 790-796.

46) Nomoto, A., Detjer, B., Pozzati, R., & Wimmer, E. (1977) Nature (London) **268**, 208-213.

47) Pettersson, R.F., Ambros, V., & Baltimore, D. (1978) J. Virol) **27**, 357-365.

48) Takegani, T., Kuhn, R.J., Anderson, C.W., & Wimmer, E. (1983) Proc) Natl. Acad. Sci. USA **80**, 7447-7451.

49) Crawford, N.M. & Baltimore, D. (1983) Proc. Natl. Acad. Sci. USA **80**, 7452-7455.

50) Onata, T., Kohara, M., Sakai, Y., Kaneda, A., Imura, N., & Nomoto, A) (1984) Gene **32**, 1-10.

51) Strauss, E.G. & Strauss, J.H. (1983) Curr. Top. Micro. Immun. **105**, 1-98.

52) Sarnow, P., Bernstein, H.D., & Baltimore, D. (1986) Proc. Natl. Acad) Sci. USA **83**, 571-575.

53) Mizutani, S. & Colonno, R.J. (1985) J. Virol **56**, 628-632.

54) Semler, B.L., Dorner, A.J., & Wimmer, E. (1984) Nucleic Acids Res) **12**, 5123-5141.

55) Meyer, M., Hemmer, O., & Fritsch, C. (1984) J. Gen. Virol. **65**, 1575-1583.

56) van der Werf, S., Bradley, J., Wimmer, E., Studier, F.W., & Dunn, J.J) (1986) Proc. Natl. Acad. Sci. USA **83**, 2330-2334.

57) Rice, E.M., Lenches, E.M., Eddy, S.R., Shin, S.J., Sheets, R.L., & Strauss, J.H. (1985) Science **229**, 726-733.

58) Brown, T.D.K., Boursnell, M.E.G., Binns, M.M., & Tonley, F.M. (1986) J) Gen. Virol. **67**, 221-228.

59) Lai, M.M.C., Baric, R.S., Brayton, P.R., & Stohlman, S.A. (1984) Proc) Natl. Acad. Sci. USA **81**, 3626-3630.

60) Spaan, W., Delius, H., Skinner, M.A., Armstrong, J., Rottier, P., Smeekens, S., Siddell, S.G., & van der Zeijst, B. (1983) Adv. Exp. Biol) **173**, 173-186.

61) Ou, J.H., Trent, D.W., & Strauss, J.H. (1982) J. Mol. Biol. **156**, 719-730.

62) Ou, J.H., Strauss, E.G., & Strauss, J.H. (1983) J. Mol. Biol. **168**, 1-15.

63) Lewis, R., Weiss, B.G., Tsiang, M., Huang, A., & Schlesinger, S. (1986) Cell **44**, 137-145.

64) Sawicki, D.L. & Sawicki, S.G. (1980) J. Virol. **34**, 108-118.

65) Sawicki, S.G., Sawicki, D.L., Keranen, S., & Kaariainen, L. (1981) J) Virol. **39**, 348-358.

66) Sawicki, S.G., Sawicki, D.L., Kaariainen, L., & Keranen, S. (1981) Virology **115**, 161-172.

67) Sawicki, S.G. & Sawicki, D.L. (1986) Virology **151**, 339-349.

68) Sawicki, S.G. & Sawicki, D.L. (1986) J. Virol. **57**, 328-334.

69) Frey, T.K., Gard, D.L., & Strauss, J.H. (1979) J. Mol. Biol. **132**, 1-18.

70) Sawicki, D.L. & Gomatos, P.J. (1976) J. Virol. **20**, 446-464.

71) Wengler, G. & Wengler, G. (1975) Virology **66**, 322-326.

72) Monroe, S.S. & Schlesinger, S. (1983) Proc. Natl. Acad. Sci. USA **80**, 3279-3283.

73) Banerjee, A.K. (1980) Microbiol. Rev. **44**, 175-205.

74) Ishida, I., Simizu, B., Koizuni, S., Oya, A., & Yamada, M. (1981) Virology **108**, 13-20.

75) Brayton, P.R., Lai, M.M.C., Patton, C.D., & Stohlman, S.A. (1982) J) Virol. **42**, 847-853.

76) Lai, M.M.C., Baric, R.S., Brayton, P.R., & Stohlman, S.A. (1984) Adv) Exp. Med. Biol. **173**, 197-200.

77) Kingsbury, D.W. (1985) In *Virology* Fields, B.N., ed. Raven Press, New York, 1157-1178.

78) Compans, R.W. & Bishop, D.H.L. (1985) Curr. Topics Microbiol. Immun) **114**, 153-175.

79) Bishop, D.H.L. (1985) In *Virology* Fields, B.N., ed. Raven Press, New York, 1083-1110.

80) Bishop, D.H.L. & Smith, M.S. (1977) In *The Molecular Biology of Animal Viruses* Nyak, D.P., ed. Dekker, New York, 167-315.

81) Pennington, T.H. & Pringle, C.R. (1978) In *Negative Strand Viruses and the Host Cell* Mahy, B.W.J. & Berry, R.D., eds. Academic Press, London, 457-464.

82) Mellon, M.G. & Emerson, S.U. (1978) J. Virol. **27**, 560-567.

83) Emerson, S.U. (1985) In *Virology* Fields, B.N., ed. Raven Press, New York, 1119-1132.

84) Hsu, C.-H., Morgan, E.M., & Kingsburg, D.W. (1982) J. Virol. **43**, 104-112.

85) Kingsford, L. & Emerson S.U. (1980) J. Virol. **33**, 1097-1105.

86) Clinton, G.M., Burge, B.W., & Huang, A.S. (1978) J. Virol. **27**, 340-346.

87) Fiszman, M., Leaute, J.-B., Chany, C., & Girard, M. (1974) J. Virol) **13**, 801-808.

88) Wilson, T. & Lenard, J. (1981) Biochemistry **20**, 1349-1354.

89) Hill, V.M., Marnell, L., & Summers, D.F. (1981) Virology **113**, 109-118.

90) Blumberg, B.M. & Kolakofsky, D. (1981) J. Virol. **40**, 568-576.

91) Blumberg, B.M., Leppert, M., & Kolakofsky, D. (1981) Cell **23**, 837-845.

92) Kurilla, M.G., Cabradilla, C.D., Holloway, B.P., & Keene, J.D. (1984) J) Virol. **50**, 773-778.

93) Kurilla, M.G. & Keene, J.D. (1983) Cell **34**, 837-845.

94) Leppert, M., Rittenhouse, L., Perrault, J., Summers, D.F., & Kolakofsky, D.F. (1979) Cell **18**, 735-747.

95) Wilusz, J. & Keene, J.D. (1984) Virology **135**, 65-73.

96) Keene, J.D., Schubert, M., & Lazzarini, R.A. (1980) J. Virol. **33**, 789-794.

97) Kurilla, M.G., Stone, H.O., & Keene, J.D. (1985) Virology **145**, 203-212.

98) Robertson, J.S. (1979) Nucleic Acids Res. **6**, 3745-3757.

99) Braam, J., Ulmanen, I., & Krug, R.M. (1983) Cell **34**, 609-618.

100) Mahy, B.W.J., Barrett, T., Nichol, S.T., Penn, C.R., & Wolstenholme, A.J) (1981) In *The Replication of Negative Strand Viruses* Bishop, D.H.L. & Compans, R.W., eds. Elsevier/North-Holland, New York, 379-394.

101) Herz, C., Stavnezer, E., Krug, R.M., & Gurney, T. (1981) Cell **26**, 391-400.

102) Smith, G.L. & Hay, A.J. (1982) Virology **118**, 96-108.

103) Petri, T., Meier-Ewert, H., & Compans, R.W. (1979) J. Virol) **32**, 1037-1040.

104) Ulmanen, I., Broni, B.A., & Krug, R.M. (1981) Proc. Natl. Acad. Sci. USA **78**, 7355-7359.

105) Robertson, J.S., Caton, A.J., Schubert, M., & Lazzarini, R.A. (1981) In *The Replication of Negative Strand Viruses* Bishop, D.H.L. & Compans, R.W., eds. Elsevier/North Holland, New York, 303-308.

106) Hay, A.J., Skehel, J.J., & McCauley, J. (1982) Virology **116**, 517-522.

107) Eshita, Y., Ericson, B., Romanowski, V., & Bishop, D.H.L. (1985) J. Virol) **55**, 681-689.

108) Patterson, J.L., Holloway, B., & Kolakofsky, D. (1984) J. Virol) **52**, 215-222.

109) Milne, R.G. & Francki, R.I.B. (1984) Intervirology **22**, 72-76.

110) Silverstein, S.C., Astell, C., Levin, D.H., Schonberg, M., & Acs, G) (1972) Virology **47**, 797-806.

111) Shatkin, A.J., Sipe, J.D., & Loh, P.C. (1968) J. Virol. **2**, 986-991.

112) Muthukrishnan, S. & Shatkin, A.J. (1975) Virology **64**, 96-105.

113) Furuichi, Y., Muthukrishnan, S., & Shatkin, A.J. (1975) Proc. Natl. Acad) Sci. USA **72**, 742-745.

114) Chow, N.-L. & Shatkin, A.J. (1975) J. Virol. **15**, 1057-1064.

115) Shatkin, A.J. & Kozak, M. (1983) In *The Reoviridae* Joklik, W.K., ed) Plenum Press, New York, 79-106.

116) Silverstein, S.C., Christman, J.K., & Acs, G. (1976) Ann. Rev. Biochem) **45**, 375-408.

117) Tyler, K.L. & Fields, B.N. (1985) In *Virology* Fields, B.N., ed); Raven Press, New York, 823-862.

118) Sakuma, S. & Watanabe, Y. (1972) J. Virol. **10**, 628-638.

119) Zweerink, H.J. (1974) Nature (London) **247**, 313-315.

120) Zarble, H. & Millward, S. (1983) In *The Reoviridae* Joklik, W.K., ed) Plenum Press, New York, 108-196.

121) Nemeroff, M.E. & Bruenn, J.A. (1986) J. Virol. **57**, 754-758.

122) Van Etten, J.L., Burbank, D.E., Cuppels, D.A., Lane, L.C., & Vidaver, A.K) (1980) J. Virol. **33**, 769-773.

123) Buck, K.W. (1975) Nucleic Acids Res. **2**, 1889-1902.

124) Ratti, G. & Buck, K.W. (1978) Nucleic Acids Res. **5**, 3843-3854.

125) Branch, A.D. & Robertson, H.D. (1984) Science **223**, 450-455.

126) Buzayan, J.M., Gerlach, W.L., Bruening, G., Keese, P., & Gould, A.R) (1986) Virology **151**, 186-199.

127) Gerlach, W.L., Buzayan, J.M., Schneider, I.R., & Bruening, G. (1986) Virology **151**, 172-185.

128) Robertson, H., Rosen, D.L., & Branch, A.D. (1985) Virology **142**, 441-447.

129) Kiberstis, P.A., Haseloff, J., & Zimmern, D. (1985) EMBO J. **4**, 817-827.

130) Hutchins, C.J., Keese, P., Visvader, J.E., Rathjen, P.D., McInnes, J.L., & Symons, R.H. (1985) Plant Mol. Biol. **4**, 293-304.

131) Collmer, C.W., Hadidi, A., & Kapen, J.M. (1985) Proc. Natl. Acad. Sci. USA **82**, 3110-3114.

132) Watson, N., Gurevitz, M., Ford, J., & Apirion, D. (1984) J. Mol. Biol) **172**, 301-323.

133) Price, J.V. & Cech, T.R. (1985) Science **228**, 719-722.

134) Cech, T.R. (1986) Cell **44**, 207-210.

135) Acheson, N.H. (1981) In *The Molecular Biology of Tumor Viruses: Second Edition, Part 2. DNA Tumor Viruses* Tooze, J., ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 125-204.

136) Pope, J.H. & Rowe, W.D. (1964) J. Exp. Med. **120**, 121-128.

137) Feunteun, J., Sompayrac, L., Fluck, M., & Benjamin, T.L. (1976) Proc) Natl. Acad. Sci. USA **73**, 4169-4173.

138) Dulbecco, R., Hartwell, L., & Vogt, M. (1965) Proc. Natl. Acad. Sci. USA **53**, 403-408.

139) Hiscott, J.B. & Defendi, V. (1979) J. Virol. **30**, 590-599.

140) Hiscott, J.B. & Defendi, V. (1981) J. Virol. **37**, 802-812.

141) Tijan, R. & Robbins, A. (1979) Proc. Natl. Acad. Sci. USA **76**, 610-615.

142) Griffin, J.D., Spangler, G., & Livingston, D.M. (1979) Proc. Natl. Acad) Sci. USA **76**, 2610-2614.

143) McCormick, F. & Harlow, E. (1980) J. Virol. **34**, 213-224.

144) Linzer, D.I.H. & Levine, A.J. (1979) Cell **17**, 43-52.

145) DeLucia, A., Lewton, B., Tijan, R., & Tegtmeyer, P. (1983) J. Virol) **46**, 143-150.

146) Stringer, J.R. (1982) Nature (London) **296**, 363-366.

147) Temen, D., Haines, L., & Livingston, D.M. (1982) J. Mol. Biol) **157**, 473-492.

148) Tijan, R. (1978) Cell **13**, 165-179.

149) Margolskee, R.F. & Nathans, D. (1984) J. Virol. **49**, 386-393.

150) Ryder, K., Silver, S., DeLucia, A.L., Fanning, E., & Tegtmeyer, P. (1986) Cell **44**, 719-725.

151) Zahn, K. & Blattner, F.R. (1985) Nature (London) **317**, 451-453.

152) Murakani, Y., Wobbe, C.R., Weissbach, L., Dean, F.B., & Hurwitz, J. (1986) Proc. Natl. Acad. Sci. USA **83**, 2869-2873.

153) Chou, J., Avila, J., & Martin, R. (1974) J. Virol **14**, 116-128.

154) Stahl, H. & Knippers, R. (1983) J. Virol. **47**, 65-76.

155) Hay, R. & DePamphilis, M. (1982) Cell **28**, 167-179.

156) Tapper, D. & DePamphilis, M. (1978) J. Mol. Biol. **120**, 401-412.

157) DePamphilis, M. & Wassarman, P.M. (1982) In *Organization and Replication of Viral DNA* Kaplan, A.S., ed. CRC Press, Boca Raton, 37-114.

158) Wobbe, C.R., Dean, F., Weissbach, L., & Hurwitz, J. (1985) Proc. Natl) Acad. Sci. USA **82**, 5710-5714.

159) Kaiser, D. (1971) In *The Bacteriophage Lambda* A.D. Hershey, ed.

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 195-210.

160) Kornberg, A. (1974) *DNA Replication* W.H. Freeman, San Francisco.

161) Kornberg, A. (1982) *1982 Supplement to DNA Replication* W.H. Freeman, San Francisco.

162) Tessman, E.S. & Tessman, I. (1978) In *The Single-Stranded DNA Phages* Denhardt, D.T., Dressler, D., & Ray, D.S., eds. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 9-29.

163) Ben-Porat, T. (1982) In *Organization and Replication of Viral DNA* Kaplan, A.S., ed. CRC Press, Boca Raton, 147-172.

164) Jacob, R.J. & Roizman, B. (1977) J. Virol. **23**, 394-411.

165) Roizman, B. & Batterson, W. (1985) In *Virology* Fields, B.N., ed) Raven Press, New York, 497-526.

166) Stanley, J. (1985) Adv. Virus Res. **30**, 139-177.

167) Harrison, B.D. (1985) Ann. Rev. Phytopathol. **23**, 55-82.

168) Mosig, G. (1983) In *Bacteriophage T4* Matthews, C.K., Kutter, E.M., Mosig, G., & Berget, P.B., eds. American Society for Microbiology, Washington, D.C., 120-130.

169) Murti, K.G., Goorha, R., & Granoff, A. (1985) Adv. Virus Res. **30**, 1-19.

170) Jackson, E.N., Jackson, D.A., & Deans, R.J. (1978) J. Mol. Biol) **118**, 365-388.

171) Goorha, R. (1982) J. Virol. **43**, 519-528.

172) Willis, D.B., Goorha, R., & Granoff, A. (1984) J. Virol. **49**, 86-91.

173) Cairns, J. (1960) Virology **11**, 603-623.

174) Pennington, J.H. & Follett, E.A. (1974) J. Virol. **13**, 488-493.

175) Prescott, D.M., Kates, J., & Kirkpatrick, J.B. (1971) J. Mol. Biol. **59**, 505-508.

176) Panicali, D. & Paoletti, E. (1982) Proc. Natl. Acad. Sci. USA **79**, 4927-4931.

177) Mackett, M., Smith, G.L., & Moss, B. (1982) Proc. Nat. Acad. Sci. USA **79**, 7415-7419.

178) Smith, G.L. & Moss, B. (1983) Gene **25**, 21-28.

179) McFadden, G. & Dales, S. (1982) In *Organization and Replication of Viral DNA* Kaplan, A.S., ed. CRC Press, Boca Raton, 174-190.

180) Moss, B., Winters, E., & Jones, E.V. (1983) In *Proceedings of the UCLA Symposium: Mechanisms of DNA Replication and Recombination* Cozzarelli, N.R., ed. Alan R. Liss, Inc., New York, 449-461.

181) Moyer, R.W. & Graves, R.L. (1981) Cell **27**, 391-401.

182) Cavalier-Smith, T. (1974) Nature (London) **250**, 467-470.

183) Forte, M.A. & Fangman, W.L. (1979) Chromosoma **72**, 131-150.

184) Berns, K.I. & Hauswirth, W.W. (1984) In *The Parvoviruses* Berns, K.I., ed. Plenum Press, New York, 1-31.

185) Hauswirth, W.W. (1984) In *The Parvoviruses* Berns, K.I., ed. Plenum Press, New York, 129-152.

186) Cukor, G., Blacklow, N.R., Hoggan, M.D., & Berns, K.I. (1984) In *The Parvoviruses* Berns, K.I., ed. Plenus Press, New York, pp. 33-66.

187) Laughlin, C.A., Jones, N., & Carter, B.J. (1982) J. Virol. **41**, 868-876.

188) Ostrove, J.M. & Berns, K.I. (1980) Virology **104**, 502-505.

189) Janik, J.E., Huston, M.M., & Rose, J.A. (1981) Proc. Natl. Acad. Sci. USA **78**, 1925-1929.

190) Carter, B., Marcus, C., Laughlin, C., & Ketner, G. (1983) Virology **126**, 505-516.

191) Richardson, W.D. & Westphall, H. (198) Cell **27**, 131-141.

192) Jay, F.T., Laughlin, C.A., & Carter, B.J. (1981) Proc. Natl. Acad. Sci. USA **78**, 2927-2931.

193) Meyers, M.W. & Carter, B.J. (1981) J. Biol. Chem. **256**, 567-570.

194) Handa, H. & Carter, B.J. (1979) J. Biol. Chem. **254**, 6603-6610.

195) Srivastava, A., Lusby, E.W., & Berns, K.I. (1983) J. Virol. **45**, 555-564.

196) Berns, K.I., Samulski, R.J., Srivastava, A., & Muzyczka, N. (1983) In *Proceedings of the UCLA Symposium, Mechanisms of DNA Replication and Recombination* Cozzarelli, N.R., ed. Alan R. Liss, Inc., New York, 353-365.

197) Samulski, R.J., Berns, K.I., Tan, M., & Muzyczka, N. (1982) Proc. Natl. Acad. Sci. USA **78**, 2077-2081.

198) Fife, K.H., Berns, K.I., & Murray, K. (1977) Virology **78**, 475-487.

199) Lusby, E., Bohenzky, R., & Berns, K.I. (1981) J. Virol. **37**, 1083-1086.

200) Rhode, S.L. & Paradiso, P.R. (1983) J. Virol. **45**, 173-184.

201) Astell, C.A., Thompson, M., Chow, M.B., & Ward, D.C. (1983) Cold Spring Harbor Symp. Quant. Biol. **47**, 751-762.

202) Wobbe, C.R. & Mitra, S. (1985) Proc. Natl. Acad. Sci. USA **82**, 8335-8339.

203) Walter, S., Richards, R., & Armentrout, R.W. (1980) Biochim. Biophys. Act) **607**, 420-431.

204) Kolleck, R., Tseng, B.Y., & Goulian, M. (1982) J. Virol. **41**, 982-989.

205) Goulian, M., Kollek, R., Revie, D., Burhans, W., Carton, C., & Tseng, B. (1983) In *Proceedings of the UCLA Symposium: Mechanisms of DNA Replication and Recombination* Cozzarelli, N.R., ed. Alan Liss, Inc., New York, 367-379.

206) Baroudy, B.M., Venkatesan, S., & Moss, B. (1983) Cold Spring Harbor Symp. Quant. Biol. **47**, 723-729.

207) Baroudy, B.M., Venkatesan, S., & Moss, B. (1982) Cell **28**, 315-324.

208) Wittek, R. & Moss, B. (1980) Cell **21**, 277-284.

209) Baroudy, B.M. & Moss, B. (1982) Nucleic Acids Res. **10**, 5673-5679.

210) Pickup, D.J., Bastia, D., Stone, H.O., & Joklik, W.K. (1982) Proc. Natl. Acad. Sci. USA **79**, 7112-7116.

211) Pogo, B.G.T., Berkowitz, E.M., & Dales, S. (1984) Virology **132**, 436-444.

212) Pogo, B.G.T., O'Shea, M., & Freimuth, D. (1981) Virology **108**, 241-248.

213) Esteban, M. & Holowczak, J.A. (1977) Virology **82**, 308-322.

214) Mocarski, E.S. & Roizman, B. (1982) Proc. Natl. Acad. Sci. USA **79**, 5626-5630.

215) Spaete, R.R. & Frenkel, N. (1982) Cell **30**, 295-304.

216) Stow, N.D. (1982) EMBO J. **1**, 863-867.

217) DeLange, A.M. & McFadden, G. (1986) Proc. Natl. Acad. Sci. USA **83**, 614-618.

218) Pogo, B.G.T. (1980) Virology **101**, 520-524.

219) Pogo, B.G.T. (1977) Proc. Natl. Acad. Sci. USA **74**, 1739-1742.

220) Esteban, M. & Holowczak, J.A. (1977) Virology **78**, 57-75.

221) Moyer, R.W. & Graves, R.L. (1981) Cell **27**, 391-401.

222) Wittek, R. (1982) Experientia **38**, 285-297.

223) Holowczak, J. (1982) Curr. Topics Microbiol. Immunol. **97**, 27-79.

224) DeLange, A.M., Futcher, B., Morgan, R., & McFadden, G. (1984) Gene 27, 13-21.

225) Merchlinsky, M. & Moss, B. (1986) Cell **45**, 879-884.

226) Sussenbach, J.S. & van der Vliet, P.C. (1983) Curr. Topics Microbiol. Immunol. **109**, 53-73.

227) Tamanoi, F. & Stillman, B.W. (1983) Curr. Topics Microbiol. Immunol. **109**, 75-87.

228) Friefeld, B.R., Lichy, J.H., Field, J., Gronostajski, R.M., Guggenheimer, R.A., Krevolin, M.D., Nagata, K., Hurwitz, J., & Horwitz, M.S. (1984) Curr. Topics Microbiol. Immunol. **110**, 221-255.

229) Futterer, J. & Winnacker, E.-L. (1984) Curr. Topics Microbiol. Immunol. **111**, 41-64.

230) Salas, M. (1983) Curr. Topics Microbiol. Immunol. **109**, 89-106.

231) Desiderio, S.V. & Kelly, T.J., Jr. (1981) J. Mol. Biol. **145**, 319-337.

232) Challberg, M.D. & Kelly, T.J., Jr. (1981) J. Virol. **38**, 272-277.

233) Ruben, M., Bacchetti, S., & Graham, F. (1983) Nature (London) **301**, 172-174.

234) Graham, F. (1984) EMBO J. **3**, 2917-2922.

235) Tamanoi, F. & Stillman, B.W. (1982) Proc. Natl. Acad. Sci. USA **79**, 2221-2225.

236) DeJong, P.J., Kwant, M.M., van Driel, W., Janz, H.S., & van der Vliet, P.C) (1983) Virology **124**, 45-58.

237) Lally, C., Dorpen, T., Groger, W., Antoine, G., & Winnacker, E.L. (1984) EMB) J. **3**, 333-337.

238) van Bergen, B.G.M., van der Ley, P.A., van Driel, W., van Mansfeld, A.D.M., & van der Vliet, P.C. (1983) Nucleic Acids Res. **11**, 1975-1989.

239) Tamanoi, F. & Stillman, B.W. (1983) Proc. Natl. Acad. Sci. USA **80**, 6446-6450.

240) Rijnders, A.W.M., van Bergen, B.G.M., van der Vliet, P.C., & Sussenbach, J.S) (1983) Nucleic Acids Res. **11**, 8777-8789.

241) Lichy, J.H., Field, J., Horwitz, M.S., & Hurwitz, J. (1982) Proc. Natl. Acad. Sci. USA **79**, 5225-5229.

242) Nagata, K., Guggenheimer, R.A., & Hurwitz, J. (1983) Proc. Natl. Acad. Sci) USA **80**, 4266-4270.

243) Nagata, K., Guggenheimer, R.A., Enomoto, T., Lichy, J.H., & Hurwitz, J. (1982) Proc. Natl. Acad. Sci. USA **79**, 6438-6442.

244) Ikeda, J.-E., Enomoto, T., & Hurwitz, J. (1982) Proc. Natl. Acad. Sci., USA **79**, 2442-2446.

245) Guggenheimer, R.A., Nogata, K., Field, J., Lindenbraun, J., Gronostajsky, R.M., Horwitz, M.S., & Hurwitz, J. (1983) In *Proceedings of the UCLA Symposium: Mechanisms of DNA Replication and Recombination* Cozzarelli, N.R., ed.) Alan R. Liss, Inc., New York, 395-421.

246) Blanco, L. & Salas, M. (1985) Proc. Natl. Acad. Sci. USA **82**, 6404-6408.

247) Garcia, J.A., Penalva, M.A., Blanco, L., & Salas, M. (1984) Proc. Natl. Acad. Sci. USA. **81**, 5374-5378.

248) Weissberg, R.A., Gottesman, S., & Gottesman, M.E. (1977) In *Comprehensive Virology, Vol. 8* Frankel-Conrat, H. & Wagner R.R., eds. Plenum Press, New York, 197-258.

249) Stringer, J.R. (1982) Nature (London) **296**, 363-366.

250) Topp, W.C., Lane, D., & Pollack, R. (1981) In *Molecular Biology of Tumor Viruses: DNA Tumor Viruses 2nd Ed.* Tooze, J., ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 205-296g.

251) Chias, W. & Rigby, P.W.J. (1981) Proc. Natl. Acad. Sci. USA **78**, 6638-6642.

252) Conrad, S.E., Liu, C.-P., & Botchan, M. (1982) Science **218**, 1223-1225.

253) Sambrook, J., Greene, R., Stringer, J., Mitcheson, T., Hu, S.-L., & Botchan, M. (1979) Cold Spring Harbor Symp. Quant. Biol. **44**, 569-583.

254) Merchlinsky, M.J., Tattersall, P.J., Leary, J.J., Cotmore, S.F., Gardiner, E.M., & Ward, D.C. (1983) J. Virol. **47**, 227-232.

255) Berns, K.I., Chung, A.K.-M., Ostrove, J.M., & Lewis, M. (1982) In *Virus Persistence* Mahy, B.W.J., Mirson, A.C., & Darby, G.K., eds. Cambridge University Press, Cambridge, UK, 249-265.

256) Samulski, R.J., Srivastava, A., Berns, K.I., & Muzyczka, N. (1983) Cell **33**, 135-143.

257) Doerfler, W., Gahlmann, R., Stabel, S., Deuring, R., Lichtenberg, U., Schulz, M., Eick, D., & Leisten, R. (1983) Curr. Topics Microbiol. Immunol. **109**, 193-228.

258) Hanahan, D. & Gluzman, Y. (1984) Mol. Cell. Biol. **4**, 302-309.

259) van Doren, K., Hanahan, D., & Gluzman, Y. (1984) J. Virol. **50**, 606-614.

260) Koch, S., Freytag von Loringhoven, A., Kahmann, R., Hofschneider, P.H., & Koshy, R. (1984) Nucleic Acids Res. **12**, 6871-6886.

261) Dejean, A., Brechot, C., Tiollais, P., & Wain-Hobson, S. (1983) Proc. Natl. Acad. Sci. USA **80**, 2505-2509.

262) Mizusawa, H., Taira, M., Yaginuma, K., Kobayashi, M., Yoshida, E., & Koike, K. (1985) Proc. Natl. Acad. Sci. USA **82**, 208-212.

263) Yaginuma, K., Kobayashi, M., Yoshida, E., & Koike, K. (1985) Proc. Natl. Acad. Sci. USA **82**, 4458-4462.

264) Bernstein, L.B., Mount, S.M., & Weiner, A.M. (1983) Cell **32**, 461-472.

265) Mizuuchi, K. (1983) Cell **35**, 785-794.

266) Higgins, N.P., Moncecchi, D., Howe, M.M., Manlapaz-Ramos, P., & Olivera, B.M) (1983) In *Proceedings of the UCLA Symposium: Mechanisms of DNA Replication and Recombination* Cozzarelli, N.R., ed. Alan R. Liss, Inc., New York, 187-201.

267) Craigie, R. & Mizuuchi, K. (1985) Cell **41**, 867-876.

268) Craigie, R. & Mizuuchi, K. (1986) Cell **45**, 793-800.

269) Craigie, R., Mizuuchi, M., & Mizuuchi, K. (1984) Cell **39**, 387-394.

270) Mizuuchi, K. (1984) Cell **39**, 395-404.

271) Chaconas, G., Gloor, G., & Miller, J.L. (1985) J. Biol. Chem. **260**, 2662-2669.

272) Groenen, M.A.M., Timmers, E., & van de Putte, P. (1985) Proc. Natl. Acad. Sci. USA **82**, 2087-2091.

273) Craigie, R., Arndt-Jovin, D.J., & Mizuuchi, K. (1985) Proc. Natl. Acad. Sci) USA **82**, 7570-7574.

274) Harshey, R.M. & Bakhari, A.I. (1983) J. Mol. Biol. **167**, 427-441.

275) Varmus, H.E. & Swanstrom, R. (1982) In *Molecular Biology of Tumor Viruses: RNA Tumor Viruses* Weiss, R.A., Teich, N., Varmus, H.E., & Coffin, J.M., eds. Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, 369-512.

276) Lowy, D.R. (1985) in *Virology* Fields, B.N., ed. Raven Press, New York, 235-263.

277) Champoux, J.J., Gilboa, E., & Baltimore, D. (1984) J. Virol. **49**, 686-691.

278) Resnick, R., Omer, C.A., & Faras, A.J. (1984) J. Virol. **51**, 813-821.

279) Smith, J.K., Cywinski, A., & Taylor, J.M. (1984) J. Virol. **49**, 200-204.

280) Smith, J.K. & Taylor, J.M. (1984) J. Virol. **52**, 314-319.

281) Taylor, J.M. & Hsu, T.W. (1980) J. Virol. **33**, 531-534.

282) Shimotohno, K. & Temin, H.M. (1980) Proc. Natl. Acad. Sci. USA **77**, 7357-7361.

283) Panganiban, A.T. & Temin, H.M. (1983) Nature (London) **306**, 155-160.

284) Misra, T.P., Grandgenett, D.P., & Parsons, J.T. (1982) J. Virol. **44**, 330-343.

285) Varmus, H.E., Padgett, T., Healsey, S., Simon, G., & Bishop, J.M. (1977) Cel) **11**, 307-309.

286) Miller, R.H. & Robinson, W.S. (1986) Proc. Natl. Acad. Sci. USA **83**, 2531-2535.

287) Toh, H., Hyashida, H., & Miyata, T. (1983) Nature (London) **305**, 827-829.

288) Summers, J. & Mason, W.S. (1982) Cell **29**, 403-415.

289) Pfeiffer, P. & Hohn, T. (1983) Cell **33**, 781-789.

290) Tuttleman, J.S., Pugh, J.C., & Summers, J.W. (1986) J. Virol. **58**, 17-25.

291) Korba, B.E., Wells, F., Tennant, B.C., Yoakum, G.H., Purcell, R.H., & Gerin, J.L. (1986) J. Virol. **58**, 1-8.

292) Molnar-Kimber, K.L., Summers, J., & Mason, W.S. (1984) J. Virol. **51**, 181-191.

293) Mason, W.S., Aldrich, C., Summers, J., & Taylor, J.M. (1982) Proc. Natl. Acad. Sci. USA **79**, 3997-4001.

294) Lien, J.-M., Aldrich, C.E., & Mason, W.S. (1986) J. Virol. **57**, 229-236.

295) Buscher, M., Reiser, W., Will, H., & Schaller, H. (1985) Cell **40**, 717-724.

296) Tiollais, P., Dejean, A., Brechot, C., Michel, M.L., Sonigo, P., & Wain-Hobson, S. (1984) In *Viral Hepatitis and Liver Disease* Vyas, G.N., Dienstag, J.L., & Hoofnagle, J.H., eds. Grune & Stratton, Inc., New York, 49-65.

297) Olszewski, N., Hagen, G., & Guilfoyle, T.J. (1982) Cell **29**, 395-402.

298) Covey, S.N., Lomonossoff, G.P., & Hull, R. (1981) Nucleic Acids Res. **9**, 6735-6747.

299) Volovitch, M., Modjtahedi, M., Yot, P., & Brun, G. (1984) EMBO J. **3**, 309-314.

300) Mazzolini, L., Bonneville, J.M., Volovitch, M., Magazin, M., & Yot, P. (1985) Virology **145**, 293-303.