REVIEW ARTICLE

RNA and Protein Synthesis in Herpesvirus-Infected Cells

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INTRODUCTION

Herpesviruses infect a wide range of vertebrates and are important human and animal pathogens: in a number of cases infection persists for very long periods, in a latent form, with occasional episodes of overt typical disease. In addition, several herpesviruses have been shown to be the causative agent of tumour formation in their normal hosts (e.g. Lucké frog tumour virus) or in related species (e.g. herpesvirus saimiri). Indirect evidence exists for the involvement of Epstein-Barr virus and herpes simplex virus type 2 (HSV-2) in human cancers. Thus there exist good reasons for the pursuit and identification of the mechanisms of herpesvirus infection and replication.

From the molecular virological standpoint, herpesviruses also offer an important system for study. This large virus has the potential to code for about one hundred genes and the identification of these gene products, their function and their control poses many interesting questions. Finally, with such a reservoir of gene functions, herpesviruses may be capable of carrying out some replication functions (e.g. DNA synthesis, perhaps) relatively independently of the host cell: any such functions, upon identification, would be useful targets for antiviral therapy.

Since herpes simplex virus type 1 (HSV-1) is the most widely studied of all the herpesviruses, the present discussion will deal largely with this virus, but other herpesviruses will be mentioned where important differences have emerged.

Herpesviruses have a complex structure, organized into an outer envelope surrounding a capsid with 162 capsomers arranged on an icosahedron. Inside this the virus DNA, a linear double strand, is arranged as a toroid wound round a protein plug (Furlong, Swift & Roizman, 1972). In addition to DNA and simple protein, the particle contains spermine and spermidine, lipid and glyco- and lipoproteins. Particle: p.f.u. ratios vary greatly, from 5:1 to 100:1 or even more with some HSV-2 strains. So far, no RNA or DNA polymerase activities have been found in particle preparations but membrane associated enzymes like protein kinase are present and may be virus-specific (Rubenstein, Gravell & Darlington, 1972). The virus DNA is infectious (Sheldrick et al. 1973). In BHK cells infected with HSV-1, virus DNA synthesis starts at about 3 h, followed by infectious virus about 1 h later: the maximum virus yield, at about 12 h, is from 50 to 100 p.f.u./cell. Much of the pool of virus proteins and DNA in the infected cell never becomes incorporated into mature virions and continued high multiplicity passage leads to accumulation of defective particles.

Several laboratories have attempted the difficult task of purification of HSV-1 virions with good yield and retention of infectivity. Probably the best of the methods was developed by Spear & Roizman (1972) and particles purified by this method contain over 30 polypeptides (Heine et al. 1974; Marsden, Crombie & Subak-Sharpe, 1976). The number of
polypeptides which are essential components as opposed to particle-associated proteins has yet to be established. Roizman et al. (1975) have recently summarized the location of many of these polypeptides in the HSV-1 particle. In addition to these structural proteins, a further 20 or so have been recognized as virus-induced by PAGE analysis of labelled infected cell polypeptides. Additional virus polypeptides may be detected by testing for enzyme activities (virus DNA polymerase, DNase, deoxypyrimidine kinase, ribonucleotide reductase and dCMP deaminase have been reported) or by using selection techniques such as DNA-affinity chromatography (Bayliss, Marsden & Hay, 1975). Comparison between the polypeptides of several strains of HSV-1 and HSV-2 (Courtney & Powell, 1975) demonstrates that a major divergence in pattern exists between the strains, a result quite compatible with their degree of genetic relatedness. This, and the fact that recombinants between HSV-1 and HSV-2 can readily be isolated (Timbury & Subak-Sharpe, 1974) gives rise to the possible physical mapping of HSV-1 and HSV-2 polypeptides in, for example, cases where recombinants can be shown to have identifiable HSV-1 polypeptides, in a background of HSV-2 polypeptides and a correspondingly identifiable HSV-1 DNA restriction enzyme fragment amongst a background of HSV-2 fragments. Preliminary data suggest that this approach can be used (N. M. Wilkie and H. S. Marsden, personal communication).

**HSV-1 genome structure**

HSV-1 DNA consists of linear duplex molecules with a mol. wt. of 95 to 100 \times 10^6 (Becker, Dym & Sarov, 1968; Kieff, Bachenheimer & Roizman, 1971; Wilkie, 1973; Delius & Clements, 1976). Following alkali denaturation, intact DNA single strands together with fragments smaller than unit length are generated (Kieff et al. 1971; Wilkie, 1973). The nature and distribution of the alkali-labile regions are still not well understood. The report that these alkali-labile regions are at unique sites in the DNA and are preferentially found in one strand (Frenkel & Roizman, 1972) has been disputed by Wilkie (1973) who found that the single strand fragments of HSV-1 DNA were distributed in an unresolved collection after high resolution gel electrophoresis. Furthermore, Wilkie (1973) showed that intact single strands contained sequences from both strands of the DNA and these self-annealed to the same extent as unfractionated DNA, suggesting that the alkali-labile bonds were distributed in a more random manner.

It is not known whether these alkali-labile regions correspond to single strand nicks or gaps or to the insertion of ribonucleotides which have been reported covalently linked to HSV-1 DNA (Hirsch & Vonka, 1974). A large proportion of the interruptions must, however, be due to single strand nicks and gaps, since they can be partially repaired by T4 DNA-ligase and, more effectively by the combination of T4 DNA-ligase and DNA polymerase (R. W. Hyman, J. E. Oakes & L. Kudler, submitted for publication; N. M. Wilkie, personal communication.

HSV-1 DNA contains terminally redundant sequences which have been estimated as about 0.5% of the genome (Grafstrom et al. 1974). In addition, from electron microscopic observations on intact single strands before and after self-reassocation, larger blocks of terminal sequences, originally thought to be about 5 \times 10^6 daltons, have been found to be repeated as internal inverted forms (Sheldrick & Berthelot, 1974). The terminal and inverted forms flank two internal unique sequences estimated to contain about 10 \times 10^6 daltons (the S sequence) and 75 \times 10^6 daltons (the L sequence) of DNA respectively. Sheldrick & Berthelot (1974) called the terminal redundant sequence at the S region TR_s, and its internal inverted form IR_s. Similarly, the redundant sequences bounding the L region were termed TR_L and IR_L (Fig. 1). They pointed out in their paper that internal
RNA and protein synthesis in herpesvirus-infected cells

Fig. 1. Diagram of the four possible arrangements which can result from inversions of the long (L) and short (S) unique regions of HSV-1 DNA. Terminal repeats (TR/L) and internal inverted repeats (IR/L) are indicated by thicker lines. The figures above the repeats in genome arrangement (1) indicate the sequence arrangement for a single strand with respect to the constant terminal redundancy which also is indicated. The genome arrangements shown represent the molecular model proposed by Sheldrick & Berthelot (1974) but have been drawn with the short unique DNA region on the left-hand side.

recombination between the redundant sequences in such a molecule could generate inversions of the entire S and L unique sequences, leading to four different arrangements of the genome shown in Fig. 1, which result from the possible combinations of unique region inversions. It is possible, however, that inversions of the unique regions could also be generated by intermolecular recombination events.

Analysis of the HSV-1 genome with restriction endonucleases (Hayward, Frenkel & Roizman, 1975; Clements, Cortini & Wilkie, 1976), partial denaturation mapping studies (Delius & Clements, 1976) and nucleic acid hybridization studies (Wilkie & Cortini, 1976) have shown that the unique sequences S and L are relatively inverted in different DNA molecules within the population, and that the four genome arrangements (Fig. 1) are present in approximately equal amounts. Physical maps for the DNA fragments produced by the Hind III, Hpa I and Xba restriction endonucleases have been constructed for the four related forms of the HSV-1 genome (Wilkie, 1976). Results from these studies and those outlined above indicate that the redundant sequences bounding S do not show complete sequence homology with the redundant sequences bounding L. TR/L were each 3·5 to 4·5 × 10^6 daltons and TR/L were each about 6·5 × 10^6 daltons. The S and L regions were approx. 10 × 10^6 daltons and 68 × 10^6 daltons respectively.

Size and location of virus transcripts

Following infection with HSV-1, there is an overall decline in the rate of RNA synthesis (Roizman, Borman & Kamali-Rousta, 1965). Wagner & Roizman (1969a) determined that the decrease in the appearance of host RNA in the cytoplasm of infected cells was due to a fall in overall RNA synthesis and to abortive processing of 45S precursor RNA which was made and methylated but was not processed to 28 and 18S ribosomal species.

Virus RNA is made in the nucleus of infected cells, where the concentration of virus
transcripts is invariably higher than in the cytoplasm and, following a 10 to 15 min lag, virus RNA can be detected in the cytoplasm (Wagner & Roizman, 1969a). Virus RNA associated with polyribosomes sedimented between 10 and 40S, while the sedimentation range of nuclear virus RNA was from 10 to 60S (Wagner & Roizman, 1969a). At early and late times post-infection, total nuclear virus transcripts are, on average, larger than virus transcripts associated with polyribosomes; however, nuclear and cytoplasmic polyadenylated RNAs are of similar size (J. R. Stringer, L. E. Holland, R. I. Swanstrom, K. Pivo, & E. K. Wagner, submitted for publication). The sizes of virus cytoplasmic transcripts are consistent with these being monocistronic messages (B. Perbal & J. B. Clements, submitted for publication).

The relative proportions of virus nuclear transcripts of different sizes were similar in pulse-labelled cells to those estimates from cells labelled for longer intervals (Wagner & Roizman, 1969b) which implies that some classes of virus RNA are made as small molecules or that cleavage of high mol. wt. precursors is highly efficient. Cytoplasmic virus RNA contained the bulk of sequences present in high mol. wt. nuclear RNA (Wagner & Roizman, 1969b) which indicates that at least some cytoplasmic transcripts are produced by cleavage of a high mol. wt. precursor.

Analysis of DNA sequences transcribed during infection

The temporal regulation of HSV-1 transcription during productive infection has been analysed extensively utilizing procedures based upon hybridizing trace amounts of labelled DNA to excess unlabelled RNA (Mayfield & Bonner, 1971). Frenkel & Roizman (1972b) have analysed the kinetics of hybrid formation in experiments where, in liquid phase, labelled virus DNA was hybridized to excess unlabelled RNA from infected cells. This experimental approach yields information on the fraction of the virus DNA transcribed, and can give an estimate of the number of virus RNA abundance classes (together with that fraction of the DNA template represented in these abundance classes) and the molar ratios of these classes.

Experiments designed to estimate the amount of the DNA template represented as early RNA (present before virus DNA synthesis) have not provided firm conclusions. Early RNA, from cells infected for 2 h, contained two classes of virus RNA species which differed 140-fold in concentration (Frenkel & Roizman, 1972b). These abundant and scarce RNA classes were transcribed from 14 and 30% of the DNA respectively (these values represent 28 and 62% of the single strand equivalent). Late RNA, isolated from cells 8 h post-infection (after the onset of virus DNA synthesis), also contained abundant and scarce classes which differed 40-fold in concentration and were complementary to 19 and 28% of virus DNA respectively. By means of abundance competition experiments, Frenkel & Roizman (1972b) showed that early abundant RNA was a sub-set of late abundant RNA. The abundant class comprised 94 and 99% of total virus-specific RNA at early and late times while the scarce class comprised 0.7 and 6.5% of the RNA at early and late times respectively (Silverstein et al. 1973).

Subsequent experiments (Kozak & Roizman, 1974), have indicated that estimates of the fraction of the genome transcribed early are multiplicity-dependent as early RNA from cells infected at a multiplicity of 10 hybridized to 20% of the DNA whereas RNA from cells infected at a multiplicity of 40 hybridized to 40% of the DNA. However, even RNA from cells infected at the higher multiplicity contained insufficient virus RNA for the hybridization reaction to reach completion. Swanstrom & Wagner (1974) have estimated that early RNA from cells infected at a multiplicity of 25 hybridized to 20% of the DNA and
RNA and protein synthesis in herpesvirus-infected cells

this estimate was similar to a value obtained using filter disc hybridization (Wagner, Swanstrom & Stafford, 1972). It seems probable that the concentration of early virus RNA sequences is a reflection both of the input virus multiplicity and the metabolic state of the host cell.

In contrast to the situation with early RNA, the various estimates of the fraction of the genome transcribed as late RNA show good general agreement. Late RNA comes from approx. 50 % of the DNA (Frenkel & Roizman, 1972b; Kozak & Roizman, 1974; Swanstrom & Wagner, 1974). The nuclear retention of late virus RNA sequences has been reported with late nuclear RNA complementary to 50 % of the DNA while RNA from cytoplasmic polyribosomes drove only 40 to 42 % of the DNA into a hybrid (Kozak & Roizman, 1974). These authors provided further evidence for sequestration of virus RNA sequences in different cell compartments as late nuclear RNA from cells in which infection was initiated and maintained in the presence of cycloheximide was complementary to 45 % of the DNA compared with a value of 10 % of the DNA obtained with cytoplasmic RNA from cycloheximide treated cells. Restricted transcription also has been reported when cells are treated with inhibitors of DNA synthesis (Wagner et al. 1972; Swanstrom & Wagner, 1974; Swanstrom, Pivo & Wagner, 1975).

It is pertinent to note that these estimates of the fraction of the virus DNA transcribed all utilize the stable virus RNA which accumulates throughout infection rather than RNA made at a particular time post-infection. This type of data gives no indication of the functionality, if any, of virus RNA classes, be they abundant or scarce. Furthermore, the interpretation of these experiments is complicated by the recent finding (Kozak & Roizman, 1975) that nuclei of infected cells contain abundant symmetric transcripts which arise from 15 % of the DNA. In contrast, cytoplasmic RNA appears to contain only small amounts of these symmetric transcripts (Kozak & Roizman, 1975).

Genome location of sequences transcribed during infection

Estimates of the proportion of virus DNA represented as RNA provide no information as to the specific genome location of the virus RNA species. Recently, a procedure has been developed which allows this information to be obtained (Southern, 1975). In this method, virus DNA is cleaved by restriction endonucleases and the resultant fragments are separated on agarose gels. After denaturation in situ, the denatured fragments are transferred to a nitrocellulose membrane which retains them in the same spatial relationship as that of the separated double-stranded fragments. Incubation of a nitrocellulose strip containing these denatured fragments with radiolabelled virus RNA enables the genome location of RNA samples to be estimated provided physical maps are available for the DNA fragments. If hybridizations are performed under conditions of DNA excess, the relative abundance of RNA hybridizing to different DNA fragments can be estimated.

Transcripts synthesized in the presence of cycloheximide have been mapped using the Southern technique (J. B. Clements, R. Watson & N. M. Wilkie, submitted for publication). By definition, this immediate early RNA must be transcribed by an unmodified host cell RNA polymerase. Results show that immediate early RNA hybridizes to restricted portions of the HSV-1 genome with an abundance of RNA hybridizing to the repeats flanking the S region. There is also hybridization to unique DNA fragments which map within the L region, to part of the repeats flanking the L region and to fragments which map just within both ends of the L region.

A striking feature of immediate early RNA was the lack of detectable hybridization to several fragments which map within the L region and which comprise approx. 40 % of
the total genome length. This restricted hybridization pattern was shown by both nuclear and cytoplasmic RNA samples. Hence, if immediate early sequences are sequestered in the nucleus relative to those in the cytoplasm (Kozak & Roizman, 1974), the concentration of these must be such that they are undetectable by the Southern technique.

In principle, these contradictions could be due to differing sensitivities between the two techniques employed. However, the blot hybridization data (J. B. Clements, R. Watson & N. M. Wilkie, submitted for publication) is consistent with the liquid hybridization data of Swanstrom & Wagner (1974) which shows one major class of immediate early RNA complementary to 20% of the DNA. Rakusanova et al. (1971) have reported the continued synthesis of a small region of pseudorabies virus DNA in the presence of cycloheximide and competition hybridization experiments revealed that this immediate early RNA contained 25% of these sequences present in early RNA. Ben-Porat, Jean & Kaplan (1974) have reported a similar finding for pseudorabies virus immediate early RNA using liquid hybridization.

These RNA mapping studies do not show the accumulation of transcripts within the nucleus in a non-functional form (as these sequences were not mobilized after cycloheximide reversal whereas cytoplasmic RNA was subsequently found to be associated with polyribosomes; Kozak & Roizman, 1974) but rather argue for export of the bulk of immediate early RNA from the nucleus to the cytoplasm.

To explain the non-functional RNA and the rapid synthesis of polypeptides from pre-existing cytoplasmic messages after cycloheximide reversal (Honess & Roizman, 1974), Roizman et al. (1975) have invoked the concept of aberrant synthesis of a second wave of transcripts in the absence of proteins specified by immediate early RNA. The RNA mapping studies would support a simpler scheme whereby transcription is restricted within the nucleus and supporting evidence for this comes from studies on the hybridization patterns of RNA made in cells infected with temperature-sensitive mutants of HSV-1 (R. Watson & J.B. Clements, submitted for publication). Certain of these mutants, with a DNA-negative phenotype under non-permissive conditions, have hybridization patterns very similar to that of immediate early RNA. All mutants of this type show a similar hybridization pattern with both nuclear and cytoplasmic RNA samples.

The RNA mapping data show that virus-specific protein synthesis is a prerequisite for the change from synthesis of immediate early RNA to that of early RNA. At the level of transcription, such a protein may serve to modify the host RNA polymerase or to alter the template activity of the virus DNA. Alternatively, a new virus-specific RNA polymerase may be synthesized. Ben-Zeev, Asher & Becker (1976) and Preston & Newton (1976) have shown that synthesis of HSV-1 RNA in nuclei isolated from cells late in infection is achieved with an α-amanitin-sensitive enzyme having similar but not identical properties to RNA polymerase II. Ben-Zeev et al. (1976) found a small amount of virus RNA synthesis resistant to α-amanitin which may be due to synthesis by RNA polymerase III.

Differences are observed when the hybridization patterns of immediate early and early RNAs are compared (J. B. Clements, R. Watson & N. M. Wilkie, submitted for publication). Most striking of these is the low abundance of early RNA hybridizing to repeats flanking the short unique region and the detection of early transcripts which hybridize to fragments comprising almost all of the genome. While the hybridization patterns give no estimate as to the proportion of a fragment transcribed, it is apparent that early transcripts are distributed throughout the genome compared with the restricted localization of immediate early RNA.

In contrast, when the hybridization patterns of early and late RNAs are compared, the
major differences are in the relative abundance of transcripts hybridizing to the various DNA fragments. In the absence of fine physical maps for the HSV-1 genome we cannot, in the comparison of the hybridization patterns of early and late RNAs, exclude on/off controls in terms of different regions being transcribed between early and late times.

Modification of virus RNA

On the basis of nitrocellulose filter binding studies, Bachenheimer & Roizman (1972) reported poly (A) sequences covalently linked to virus-specific RNA in both nuclear and cytoplasmic RNA preparations from infected cells. The size of the largest poly (A) tract linked to virus RNA was about 160 nucleotides and their results indicated that addition of poly (A) to virus-specific RNA was by a post-transcriptional mechanism. Hybridization studies (Silverstein et al. 1973) indicated that polyadenylated nuclear and polyribosomal virus RNAs were complementary to 24 and 22 % of virus DNA respectively. Abundance competition experiments indicated that these polyadenylated RNAs correspond to the abundant RNA detected late in infection. Material which did not bind to the nitrocellulose filters was assumed to be non-adenylated or minimally adenylated and the hybridization data indicated that this RNA was composed of the scarce sequences.

Recent studies, however, have shown a more complex adenylation pattern of virus-specific RNA (Silverstein et al. 1976). Several different size classes of poly (A) tracts with lengths generally less than 155 poly (A) residues were detected in RNA that did not bind to nitrocellulose filters while RNA binding to the filters contained predominantly 155 poly (A) residues. When polyadenylated RNA was separated by affinity chromatography using poly (U), three major size classes of poly (A) tracts with 30, 50 and 155 poly (A) residues were detected. The poly (A)155 class was the most abundant RNA associated with polyribosomes while free cytoplasmic RNA contained greater amounts of the poly (A)30 and poly (A)50 classes. Within the nucleus, poly (A)30 was most abundant and was the only size class detected in high mol. wt. (> 45S) nuclear RNA. There was no detectable difference in the size and distribution of these different poly (A) classes between infected and uninfected cells.

Hybridization studies indicated that virus RNA from each of these poly (A) classes hybridized with approx. 40 % of the DNA. As RNA binding to filters hybridized only to 22 % of the DNA, binding appears to be not only a function of poly (A) but also may be due to RNA secondary structure. The poly (A)155 RNA binding to nitrocellulose filters is therefore a sub-set of the total poly (A)155 population and Silverstein et al. (1976) showed that after denaturation, RNA binding to filters hybridized with 40 % of the DNA.

Although no precursor-product relationship was observed between RNA containing short and long poly (A) chains, the demonstration that high mol. wt. nuclear RNA contains only poly (A)50 indicates that adenylation is not a single step process. Silverstein et al. (1976) have suggested that poly (A)50 chains may arise by a transcriptional process similar to that suggested for generation of the short internal poly (A) stretches in HeLa cell Hn RNA (Nakazato, Edmonds & Kopp, 1974) and that further adenylation of virus RNA results from a post transcriptional mechanism.

Examination of polyadenylated and non-polyadenylated virus RNA has demonstrated the presence of ‘capped’ 5’ ends (J. B. Clements, J. Hay, & A. Shatkin, unpublished observations; E. K. Wagner, personal communication). The nature of the ‘capped’ 5’ end appears similar to that of eukaryotic messenger RNA. Use of an analogue of S-adenosyl-methionine, which inhibits in vivo methylation of RNA, has suggested that a methylation process is necessary for translation of virus RNA (B. Jacquemont, personal communication).
Control of virus polypeptide synthesis

Pulse-labelling experiments in herpesvirus infected cells using radioactive methionine at various times after infection clearly show that different polypeptides are synthesized at different times. Attempts to categorize the timing of herpesvirus polypeptide synthesis by drug treatment and release were made by Rakusanova et al. (1971) and Ben-Porat et al. (1974), who successfully demonstrated that an immediate early class of proteins was made soon after the release of a cycloheximide block in pseudorabies virus infected cells. This was more sharply defined by Honess & Roizman (1974) in HSV-1 infected cells and allowed the definition of three groups of polypeptide synthesis using the same type of treatment. The first of these groups \( \alpha \) was made, along with some host polypeptides, immediately after the removal of cycloheximide but could be distinguished from host protein since the initial rates of synthesis of \( \alpha \) polypeptides rose with the multiplicity of infection. Polypeptides in the other two groups \( \beta \) and \( \gamma \) require prior infected cell protein synthesis for their formation and differ from each other in that \( \beta \) was not made immediately upon removal of cycloheximide added before about 1 h after infection and \( \gamma \) was not immediately made on cycloheximide removal before about 2 h after infection. Also, \( \alpha \) and \( \beta \) synthesis was switched off after a period while \( \gamma \) polypeptide synthesis continued until late in infection. This last group contained the major virus structural polypeptides, while \( \alpha \) and \( \beta \) were made up from both structural and non-structural proteins.

Further work with actinomycin D and with canavanine and azetidine (Honess & Roizman, 1975) led to and supported a model of sequential ordering and coordinate regulation, in which \( \alpha \) polypeptide(s) are responsible for the switching-on of \( \beta \) polypeptides which in turn are required for the switch-off of \( \alpha \) polypeptides, with a similar scheme for \( \beta \) and \( \gamma \) proteins. It was also pointed out, that, since the \( \alpha \) mRNA was reasonably stable, the likely switch off mechanism was probably a translational one.

Powell & Courtney (1975) were also able to detect three classes of protein synthesis in cells infected with HSV-2, and these could be recognized in pulse experiments during the virus growth cycle. However, when attempts were made to use the cycloheximide block techniques outlined above, the behaviour of HSV-2 turned out to be quite different from that of HSV-1 or pseudorabies virus; immediate synthesis of early proteins could be seen, but no host synthesis resumed and the later classes of polypeptide were difficult to discern.

Ward & Stevens (1975) found that a 'late' class of polypeptide fails to be synthesized when virus DNA synthesis was inhibited by aCyt, but others have evidence for the synthesis of virion structural polypeptides and only quantitative alterations in overall polypeptide synthesis under similar drug-inhibition of DNA synthesis (Nii et al. 1968; Honess & Roizman, 1974). In addition, DNA negative \( ts \) mutants of HSV can synthesize empty particles at the non-permissive temperature (Esparza et al. 1974). Temperature-sensitive virus mutants have also been used in an approach to the overall control of HSV polypeptide synthesis (Marsden et al. 1976). Analysis of a wide range of HSV-1 mutants on PAGE after amino acid labelling reveals a complex situation. The findings would support the temporal cascade control model of Honess & Roizman (1974), possibly extending it to a more intricate series of control steps. For example, several of the Glasgow and Houston DNA negative HSV-1 mutants (Courtney & Benyesh-Melnick, 1974) are defective in switch-off of \( \alpha \) polypeptides, but synthesize some \( \gamma \) polypeptides. Mutants in one complementation group give closely related polypeptide patterns and DNA negative mutants are generally more defective than DNA positive mutants. Again, some DNA negative mutants synthesize several virus structural polypeptides. Similar data for HSV-2 mutants have recently been
described (H. S. Marsden, G. Hope, J. Hay & M. Timbury, submitted for publication). Two particularly interesting mutants, \( ts \) K of HSV-1 and \( ts \) 9 of HSV-2 produce only two or three recognizable virus polypeptides at non-permissive temperatures: whether this constitutes an 'early \( \alpha \)' class remains to be seen.

A recent development in the study of herpesvirus polypeptide synthesis and control has been the demonstration of \textit{in vitro} synthesis of proteins using RNA from infected cells (C. M. Preston, personal communication; W. Summers, personal communication). On the basis of molecular weight identity and immunological specificity, several of these proteins seem to be viral. It has also been demonstrated that RNA from cells infected with TK\(^-\) mutants of HSV-1 fails to programme the synthesis of the putative TK polypeptide (43 Kd). Finally, \textit{in vitro} products tend to emphasize the extent of post-translational modification which has been documented for herpesvirus polypeptides (Gibson & Roizman, 1974; Courtney & Powell, 1975; Spear, 1976; H. S. Marsden & M. Suh, personal communication). It should now be feasible, using fractionated RNA and hybridization to restriction enzyme fragments of virus DNA to correlate directly the expression of a particular polypeptide(s) with different regions of the DNA.

\textit{Defective particles}

There have been several demonstrations of the formation of defective particles of HSV-1 (Bronson \textit{et al.} 1973; Frenkel \textit{et al.} 1975; Schroder \textit{et al.} 1976). Typically, this DNA (from these particles) is of higher density than wild-type DNA and is resistant to cleavage by some restriction endonucleases. This had led to its characterization as multiple repeats of about \( 5 \times 10^6 \) daltons of virus DNA to form an approximately genome sized molecule (Frenkel \textit{et al.} 1975). These same defective populations over-produce ICP4 (VP W5) in infected cells and the identification of defective DNA sequences from the repeats flanking S and a small portion of S provides evidence for the expression of ICP4 from a gene located in that region of the DNA. In contrast, DNA from defective HSV-1 ANG particles has the same buoyant density as whole virus DNA and restriction enzyme analysis suggests heterogeneity in the population of genomes. Clearly, if a range of virus sequences can be effectively 'cloned' in the formation of defective particles this could be a most useful means of identifying association between specific polypeptides and known regions of virus DNA. It seems likely, however, that sequences in defective DNA will necessarily contain an initiation site for virus DNA synthesis, and this may impose restrictions on their possible genome structures.

\textit{General Conclusions}

The current evidence on control of herpesvirus polypeptide synthesis leads to the following conclusions. Virus DNA enters the cell where it is transcribed (in the nucleus) by host cell RNA polymerase II. These transcripts are derived from a restricted set of virus DNA sequences and code for a restricted set of virus polypeptides (immediate early, '\( \alpha \)' ) which are made in the cytoplasm of infected cells. Thus, transport of virus RNA from nucleus to cytoplasm must take place and this may be accompanied by processing and/or some loss of sequences. After a time, either the same or a modified host RNA polymerase, or a virus-coded RNA polymerase, in the presence of a virus-coded polypeptide, either recognizes additional promoter sites on the virus DNA, or reads through termination signals or the stability of virus message is altered; this results in the synthesis of a second class of polypeptide (early, '\( \beta \)' ) and small amounts of a third class (late, '\( \gamma \)' ). After virus DNA synthesis the level of synthesis of late, '\( \gamma \)' polypeptides rises in the final phase of protein production.
in which the relative abundance ratios of virus transcripts have been readjusted, presumably by mechanisms similar to those outlined above. The nature of the early–late transition remains obscure, but the herpes system may have a few parallels with bacteriophage T₄ which has two classes of late genes; true late genes which function only from replicated DNA and quasi-late genes which function to a limited extent from unreplicated parental DNA (Salser, Bolle & Epstein, 1970).

The concept of promoter sites in HSV DNA does, however, raise potential problems if the structure of the genome is considered. A promoter in a terminal repeat will also be present in the internal inverted form of that repeat. Assuming that both promoter sequences are functional, for each genome arrangement the RNA made will be complementary to both strands of the unique region between the promoters. Self-complementary transcripts can be generated by this mechanism provided that the initial transcript from one strand is more than half the length of this unique region. Self-complementary RNA also arises if only one of the promoters is functional. However, this requires participation of both inversions of that unique region, and again assumes that the initial transcript from one strand is more than half the length of the unique region. Symmetric transcripts could also arise from integrated virus DNA under the influence of host promoters provided different inversions of unique regions were integrated. It is not clear whether HSV-1 symmetric transcripts (Kozak & Roizman, 1975) are generated by the inherent structure of the HSV template or by strand switching which is not a consequence of the genome arrangement.

Transcripts which are initiated in a unique sequence and which terminate either within this unique region or within its flanking repeats are unaffected by the inversions. However, transcripts which initiate within one unique region and terminate within the adjacent unique region are affected by the inversion of this adjacent region, as both strands of the inverting sequence will be transcribed. Similarly, the location of termination sites will be affected by the different inversions of unique DNA regions.

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RNA and protein synthesis in herpesvirus-infected cells


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