

## Virus Synthesis and Replication: Reovirus vs. Vaccinia Virus

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Received July 12, 1979

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The strategies with which two viral genomes that consist of double-stranded nucleic acid express themselves in infected cells are compared. The reovirus genome comprises ten segments of double-stranded RNA, each of which is, in essence, a gene. Each is transcribed into plus-stranded RNA which has two functions: to serve as messenger RNA for the synthesis of the ten reovirus "primary" proteins, and to serve as template for the synthesis of minus-strands with which they remain associated, thereby giving rise to progeny double-stranded RNA. One of the most fascinating unsolved features of the reovirus multiplication cycle is the nature of the mechanism that ensures that each progeny virus particle contains a complete set of the ten individual genome RNA segments.

The vaccinia virus genome is a linear molecule of double-stranded DNA which possesses sizable terminal redundancies (up to 7 percent, depending on the strain). The vaccinia virus multiplication cycle can be divided into a well-defined early and late period. During the early period, infecting virus particles are first uncoated to cores within which some 40-50 percent of the viral genome is transcribed. These cores are then uncoated further to naked viral DNA, a process that is mediated by protein(s) translated from the "core" messenger RNA. The overall transcription pattern is highly complex and is regulated both at the transcriptional as well as at the translational level. The most profound program changes occur at the time when DNA replication begins, when the transcription of "early" messenger RNAs, some of which are translated into "early" enzymes, gives way to that of "late" messenger RNAs, most of which are translated into structural virus components.

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The purpose of this brief review is to compare the strategies with which the double-stranded RNA containing reovirus and the double-stranded DNA containing vaccinia virus express the information that they contain, and to highlight some recent advances in our ability to detect and identify type-specific virus-coded proteins. Both viruses belong to families that include some important human pathogens. The Reoviridae include the rotaviruses which cause gastroenteritis, especially in infants, which develops into a very severe disease when compounded by malnutrition and lack of medical care; the Poxviridae include smallpox virus, probably mankind's most devastating scourge through the ages, which, although on the point of being eradicated, must yet be guarded against carefully for the foreseeable future.

### REOVIRUS

#### *The Reovirion*

The genome of reoviruses, which are closely related to the rotaviruses, consists of ten segments of double-stranded (ds) RNA (Fig. 1) encased within a capsid shell that

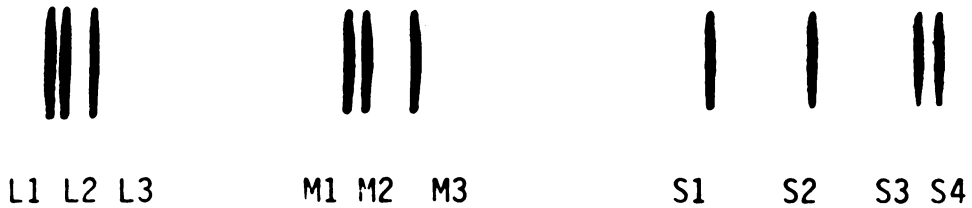


FIG. 1. Autoradiogram of a 7.5 percent polyacrylamide gel in which the ten ds RNA segments of reovirus type 3 (Dearing strain) had been electrophoresed. The direction of migration was from left to right.

consists of two protein layers [1-3]. These ten segments contain the code for ten proteins, eight of which are structural, that is, present within the virus particle, while two are nonstructural. In addition, one of the structural proteins exists in the virus particle in two forms: the form that is actually encoded in the viral genome, and a cleavage product that is some 8,000 daltons smaller [4]. The nature, size, and amount and location in virions of the reovirus-coded proteins are summarized in Table 1.

The ds RNA that is present in reovirus particles cannot express itself in cells; there are no enzymes in uninfected cells capable of transcribing ds RNA into ss RNA. Instead the ds RNA is transcribed by a transcriptase that is present in latent form in intact reovirus particles and is activated by removal of all or part of the outer capsid shell [5-8]. All attempts to isolate this transcriptase have failed. Cores transcribe ds into ss RNA very efficiently; but as soon as they are disrupted, enzyme activity disappears. This has led to the notion that the enzyme is located on and is part of the inner core surface, which would imply that the catalytic sites are fixed and that the RNA templates move past them. The ss transcripts are released through 12 hollow spikes that are distributed with icosahedral symmetry on the core surface [9]. The mechanism of the transcription process is noteworthy; it is completely conservative [7], just like the transcription of ds DNA into mRNA; that is, neither of the strands of the template appears among the transcripts. Equally noteworthy are the kinetics of transcription of each individual segment. Under optimal conditions *in vitro* the rate of transcription of all segments is the same, regardless of their size, so that equal amounts of all transcripts are formed, the frequency of transcription of each segment

TABLE I  
The Proteins Encoded by Reovirus

Species	Molecular Weight	Approximate Number of Molecules Per Virion	Location in Virion
$\lambda$ 1	155,000	100	Core
$\lambda$ 2	140,000	100	Core spike
$\lambda$ 3	140,000	12	Core (?)
$\mu$ NS	88,000	Nonstructural	—
$\mu$ 1	80,000	24	Outer shell (?)
$\mu$ 2	72,000	12	Outer shell (?)
$\mu$ 1C	72,000	550	Outer shell
$\sigma$ 1	42,000	12-24	Outer shell
$\sigma$ 2	38,000	200	Core
$\sigma$ NS	36,000	Nonstructural	—
$\sigma$ 3	34,000	900	Outer shell

being inversely proportional to its size. Since it is almost certain that the catalytic enzyme sites are fixed and that the templates move past them, this implies that the ten segments within each virus particle are unlinked. This is an important point relative to reovirus morphogenesis where one of the principal, so far unanswered questions is the nature of the mechanism that ensures that each progeny virus particle receives one of each of ten different RNA segments. One possibility would, of course, be that the segments recognize each other in some manner. However, it is known that they are not joined end-to-end, that they do not hybridize with each other to a significant extent (less than 1 percent), and that, as just pointed out, they behave as independent entities in their role as templates within virus particles.

The nature of the proteins encoded by each of the ten ds RNA segments has recently been determined both by biochemical and by genetic means [10,11]. The coding assignments are given in Table 2.

In addition to the ten segments of ds RNA, reovirions contain about 3,000 oligonucleotide molecules from 2-20 residues long, which fall into two classes. About one-half consist only of A: these are the oligoadenylates. The remainder fall into the following series: GC<sub>OH</sub>; GCU<sub>OH</sub>; GCUA<sub>OH</sub>; GCUA(U)<sub>1-4</sub>U<sub>OH</sub>; GCUA(A)<sub>1-3</sub>A<sub>OH</sub> [12]. The function of these oligonucleotides is not known. One theory that has been advanced for their presence is that they are formed during a terminal stage of morphogenesis, immediately before the virion transcriptase is inactivated (see below). At this stage, it has been postulated, there is a brief period of time when only short segments of template can be transcribed, and the products of this abortive transcription are sealed into the maturing virions [12]. This conclusion is supported by the fact that oligoadenylates are formed within the cores of nascent virions after the completion of ds RNA synthesis [13], and the fact that no ts mutants of reovirus synthesize oligoadenylates at nonpermissive temperatures (when the terminal stages of viral maturation would not proceed) [14].

### *The Reovirus Multiplication Cycle*

The overall strategy of the reovirus multiplication cycle is shown in Fig. 2. It can be divided into an early and a late period, the demarcation between them being the onset of progeny ds RNA formation. During the early period, the following functions are performed.

*Uncoating.* Reovirus is taken up into cells within phagocytic vacuoles which fuse

TABLE 2  
Nature of the Protein Encoded by Each of the Ten Segments of Reovirus ds RNA

Segment	Protein
L1	$\lambda 3$
L2	$\lambda 2$
L3	$\lambda 1$
M1	$\mu 2$
M2	$\mu 1$
M3	$\mu NS$
S1	$\sigma 1$
S2	$\sigma 2$
S3	$\sigma NS$
S4	$\sigma 3$

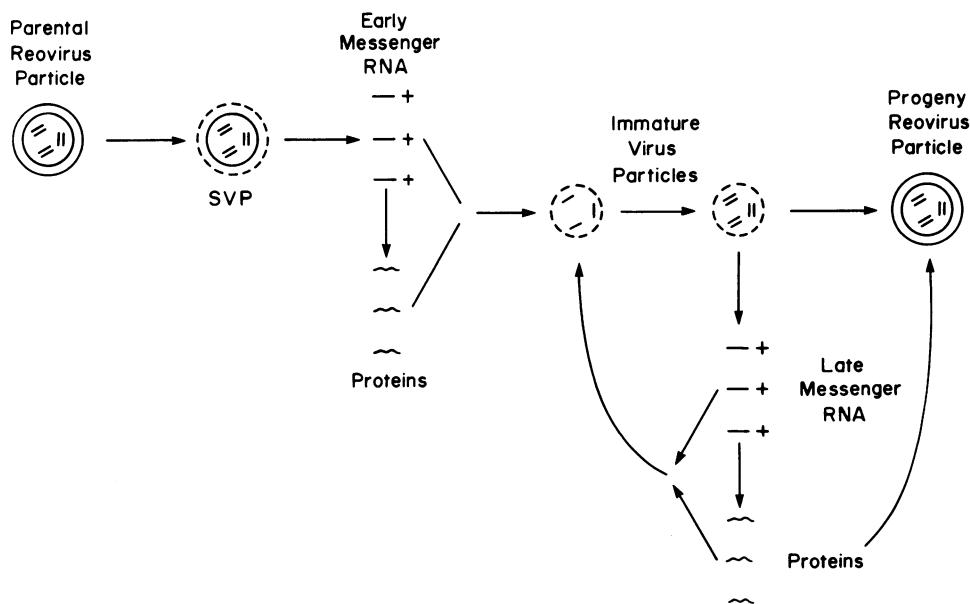


FIG. 2. The strategy of the reovirus gene expression program. For details see text.

with lysosomes within which their outer capsid shell is partially degraded by the removal of polypeptides  $\sigma 3$  and  $\sigma 1$  and an 8,000 to 12,000 dalton fragment of  $\mu$  IC [15,16]. The resulting particles, termed subviral particles (SVPs), are then liberated into the cytoplasm where they transcribe parental genome RNA into mRNA, but otherwise persist unchanged until the late stages of the infection cycle, when polypeptides  $\sigma 1$  and  $\sigma 3$  reassociate with them, and they are then liberated together with progeny virions. At no stage during the multiplication cycle does parental ds RNA exist free within the infected cell.

*The Intracellular Transcription of Reovirus RNA.* The SVPs transcribe parental ds RNA into mRNA molecules which are exactly as long as their templates; and they are capped and methylated at their 5' ends [17]. However, in distinction to most other types of mRNAs, they are not polyadenylated at their 3' ends. The frequency with which the ten ds RNA templates are transcribed is not identical with that occurring *in vitro* under optimal conditions, when the frequency of transcription is inversely proportional to the size of each segment (see above); instead, there are major deviations, especially for the larger segments which are transcribed less frequently than expected. The most probable reason for this is that the intracellular  $Mg^{++}$  and XTP concentrations are not those necessary for optimal rates of transcription.

Messenger RNA molecules synthesized during the early period of the reovirus multiplication cycle contain exclusively cap 1 ( $m^7GpppG^m pCp \dots$ ); later about one-third of the mRNA molecules possess cap 2, which contains an additional methyl group ( $m^7GpppG^m pC^m p \dots$ ) [18]. Transcripts containing cap 1 are also synthesized *in vitro* by reovirus cores and are therefore formed exclusively by enzymes present in reovirions. Whether all capping and methylating enzymes are virus-coded, or whether some are present in cores but are host-specified, is not known.

*Translation of Reovirus mRNA.* The ten species of mRNA that are synthesized during the early period are translated into polypeptides. The relative frequencies with which the various mRNA species are translated within infected cells are very similar

to those with which they are translated *in vitro*. Therefore no specific control mechanisms regulating the frequency of translation of reovirus mRNA species appear to exist; such mechanisms as there are must reside within the mRNA molecules themselves. Since it is possible to measure precisely the frequencies with which the individual species of mRNA and polypeptides are synthesized, it is possible to determine the relative efficiencies with which each species of mRNA is translated. It turns out that some species are translated more than 30 times as frequently as others (Table 3).

Impressive progress has been made recently sequencing the reovirus mRNAs; the 5'-terminal 30–40 residues of 3 s species and of 3 m species of RNA have been reported [19–21]. The first AUG which could function as an initiation codon is located from 15 to 33 residues from the 5'-terminus, depending on the RNA species.

*The Late Period.* After several hours, and presumably when a certain amount of ss RNA molecules and viral proteins has accumulated, morphogenesis commences. The first stage consists of the formation of structures that contain several reovirus-coded proteins among which the nonstructural protein  $\mu$ NS is prominent, and sets of the ten species of ss RNA. Within these particles, which are sensitive to ribonuclease, each of the ten species of mRNA is transcribed once and once only into a minus strand which remains associated with it, thereby giving rise to the progeny ds RNA molecules [22–24]. The enzyme that catalyzes this transcription has not yet been isolated, but is in all probability a form of the transcriptase that transcribes ds RNA molecules into ss RNA molecules during the early phase of the infection cycle. Nothing is known concerning the nature of the interactions that ensure that each progeny virus particle contains a complete and unique set of all ten reovirus RNA molecules; it seems likely that both protein-RNA and RNA-RNA interactions are involved, since neither alone seems sufficiently strong. The protein composition of these immature virus particles then gradually changes until particles result that resemble cores, and finally mature virions appear [25]. Throughout this morphogenetic pathway the immature virus particles transcribe the ds RNA that they contain into mRNA molecules, just like parental SVPs during the early period. In fact, since

TABLE 3  
Approximate Relative Frequencies of Transcription and Translation of the Ten Species of Reovirus mRNA *in vivo* (3)

mRNA Species	Transcription frequency	Polypeptide species	Translation frequency	Translation frequency
				Transcription frequency
l1	0.05	$\lambda$ 3	<0.02	<0.4
l2	0.05	$\lambda$ 2	0.15	3
l3	0.05	$\lambda$ 1	0.1	2
m1	0.15	$\mu$ 2	0.015	0.1
m2	0.3	$\mu$ 1 + $\mu$ 1C	1.0	3.3
m3	0.5	$\mu$ NS	0.5	1
s1	0.5	$\sigma$ 1	<0.05	<0.1
s2	0.5	$\sigma$ 2	0.2	0.4
s3	1.0	$\sigma$ NS	0.3	0.3
s4	1.0	$\sigma$ 3	0.7	0.7

there are many more immature virus particles in cells than parental particles, most (about 95 percent) of the mRNA that is formed during the infection cycle is transcribed from immature virus particles [26]. The transcriptase is active in immature virus particles until the outer capsid shell is completed during the final stage of morphogenesis, and it is presumably at that stage that the shut-off of transcriptase activity leads to the trapping within the nascent mature virus particles of the oligonucleotides, the abortive products of transcription during the final moments before it is shut off.

#### *The Nature of the Type-Specific Reovirus RNA Sequences*

Three reovirus serotypes are known, each consisting of several closely related strains. It is of great interest to determine where the RNA sequences are that differ in the genomes of strains belonging to these three serotypes, and which are the type-specific proteins.

This problem can be approached in two ways: analysis of the ability of the RNA of strains of the three serotypes to hybridize with each other, and analysis of the serological relatedness of the proteins coded by strains of the three serotypes.

It can be shown by several techniques, such as ds RNA-ds RNA or ds RNA-ss RNA hybridization, that strains of serotypes 1 and 3 are related to the extent of about 80 percent, whereas strains of serotype 2 are related to those of the other two serotypes only to the extent of about 10 percent [27,28], the criterion in each case being the resistance of hybrids to RNase under conditions when homologous hybridization is arbitrarily assigned a value of 100 percent. A more refined type of analysis is to observe the electrophoretic migration behavior of individual hybrid RNA segments. It is known that the greater the mismatch between the two strands of hybrid ds RNA molecules, the more slowly do they migrate in polyacrylamide gels relative to homologous hybrids in which both strands are derived from the same virus strains and which, by definition, exhibit perfect base pairing (100 percent homology). When this type of analysis is carried out with the RNAs of strains of serotypes 1 or 3 on the one hand and serotype 2 on the other, it is found that there is tremendous mismatching in all segments; in fact, only segments S3 and S4 will enter the 7.5 percent polyacrylamide gels in which electrophoresis is normally carried out, all others being so mismatched that they fail to enter. When a similar analysis is carried out with the RNAs of serotypes 1 and 3, the various segments are retarded to varying degrees, indicating different degrees of mismatching (Fig. 3); and we are engaged at this time in correlating the extent of mismatching as quantitated by digestion with ss RNA-specific nucleases with the extent of retardation. One segment, however, will not enter gels at all, indicating serious mismatching: this is segment S1 which codes for polypeptide  $\sigma 1$ . This segment clearly contains the most type-specific information of reoviruses.

This assessment fits in well with data that demonstrate that polypeptide  $\sigma 1$  is the primary type-specific determinant of reovirus, and that it is also the reovirus hemagglutinin and controls host-specificity and the nature of the pathogenic changes that reovirus causes [29-32]. We have recently taken this analysis further and started to quantitate the immunologic relatedness of all ten proteins coded by reovirus. The approach is as follows. We have prepared antisera to strains of the three serotypes in pathogen-free rabbits. These antisera were then used to precipitate reovirus-coded polypeptides from extracts of radioactively labelled infected cells. The immune precipitates were collected on staphylococcal A protein and the amount of each precipitated polypeptide was quantitated after polyacrylamide gel electrophoresis

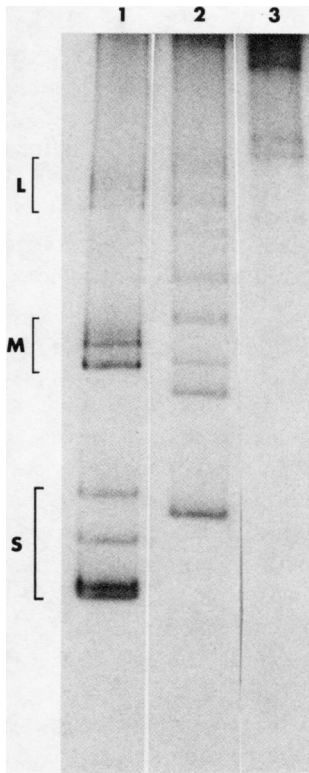


FIG. 3. Autoradiogram of a 7.5 percent polyacrylamide slab gel in which hybrid ds RNA segments of reovirus RNA had been electrophoresed. These hybrids were prepared by hybridizing radioactively labeled plus-stranded RNA with an excess of unlabeled ds RNA; in all cases shown here the ds RNA was that of reovirus type 3, and the plus-stranded RNA was that of reovirus type 3 (homologous hybrids) (slot 1), reovirus type 1 (slot 2) and reovirus type 2 (slot 3) (heterologous hybrids). Note that the heterologous hybrids of reovirus type 3/reovirus type 1 RNA are retarded relative to the homologous reovirus type 3/reovirus type 3 RNA hybrids, and that most of the heterologous reovirus type 3/reovirus type 2 RNA hybrids are retarded so severely that they are unable to enter the gel; the two hybrids that are visible are those of segments S4 and S3 [28].

and autoradiography. By using various concentrations of antisera, and by using the antisera and the extracts of infected cells in both homologous and heterologous combinations, it has been possible to quantitate the immunologic relatedness of the reovirus polypeptides. This analysis also indicated that by far the most private or type-specific of the reovirus-coded polypeptides is polypeptide  $\sigma 1$ ; for example, there is no immunologic relatedness whatsoever between the  $\sigma 1$  polypeptides of reovirus types 1 and 3, and there is only a small degree of relatedness between the  $\sigma 1$  of serotype 2 on the one hand and that of serotypes 1 and 3 on the other. As for the other reovirus-coded polypeptides, all of them show a very high degree of serologic relatedness, even those of serotype 2 on the one hand and serotypes 1 and 3 on the other. This is a most surprising result, since, as pointed out above, hybridization analysis of the RNAs of strains of serotype 2 on the one hand and serotypes 1 and 3 on the other showed very marked mismatching. It seems therefore that for all polypeptides except  $\sigma 1$ , the sequences that contain the immunologic determinants of reovirus polypeptides have been greatly conserved during evolution, while the other sequences have diverged widely; for polypeptide  $\sigma 1$ , both types of sequences have diverged extensively.

## VACCINIA VIRUS

### *The Vaccinia Virus Particle*

The genome of vaccinia virus consists of a single ds DNA molecule with a molecular weight of about 125 million. The two strands are linked covalently very close to or, most likely, at their ends; on melting, vaccinia DNA gives rise to a circle,

the circumference of which is exactly twice the length of the linear molecule. The bonds holding the two strands together are susceptible to hydrolysis by ss DNA-specific nucleases. The arrangement of genetic information in vaccinia DNA has recently begun to be explored with the preparation of restriction endonuclease maps [33]. Mapping has revealed that vaccinia DNA contains large inverted terminal repeats that differ in size from strain to strain and may be as large as 8 million daltons [34,35]. These terminal regions are subject to marked genetic instability; in stocks of uncloned virus strains the lengths of the terminal repeats are heterogeneous, and it is only upon cloning that populations of virus particles with identically sized repeats are obtained. Clearly, deletions are readily induced in these terminally repeated regions, and it has been found that the deletions at each end are always identical. This bizarre behavior raises the question as to the source of the genetic information necessary to create longer repeats, as happens when clones with short repeats are passaged repeatedly to generate virus populations in which they are again heterogeneous.

The vaccinia virus particle itself is a highly complex structure that comprises at least 50 species of polypeptides each present to the extent of more than 100 copies each; the total number of virus-coded species may well exceed 100. The vaccinia virus genome is large enough to encode this number of proteins; a ds DNA of 125 million daltons is large enough to code for 200 protein species with an average molecular weight of 35,000, even if each coding sequence is used only once. It should be noted that recent work with other systems, such as  $\phi$ X174 [36] and polyomaviruses [37], has indicated that coding sequences may well be used several times to code for different proteins.

The gross morphological features of vaccinia virus particles include a centrally located core that contains the DNA and a surrounding "outer protein coat" that is contained within a surface layer, a prominent feature of which are rodlets or tubules that are arranged in a characteristic whorled arrangement (and that display a highly regular and uniform pattern in parapoxvirus particles). The vaccinia virus core contains an arsenal of enzymes, many of which have been isolated and characterized. First there is an RNA polymerase which is absolutely essential since infecting ds DNA lodges in the cytoplasm of infected cells, where there are no enzymes capable of transcribing ds DNA into mRNA. There are also capping enzymes, a poly A polymerase (in distinction to reovirus mRNAs, vaccinia virus mRNAs are polyadenylated at their 3'-termini), a cyclic AMP-independent protein kinase, a DNA nicking-closing enzyme that relaxes both left- and right-handed superhelical DNA, two ss DNA-specific nucleases, and two DNA-dependent nucleoside triphosphatases. Many of these enzymes may be involved in the replication and packaging of DNA, and in the transcription and extrusion of nascent mRNA from cores. Vaccinia virus is also being examined for "splicing" enzymes. It has been reported that some of the RNA transcripts synthesized *in vitro* by vaccinia virus cores are very large and are "processed" as they are extruded [38]. There is, however, clear evidence from UV-transcriptional mapping studies that transcripts of vaccinia DNA are monocistronic [39].

### *The Vaccinia Virus Multiplication Cycle*

Vaccinia virus particles enter cells via phagocytic vacuoles within which they are broken down to cores which are liberated into the cytoplasm. Stripping away of the outer regions of vaccinia virus particles results in the activation of the DNA-dependent RNA polymerase which is latent in intact virus particles. Transcription of the viral genome commences within cores, almost 50 percent of the viral DNA being



transcribed. Expression of some early viral gene product appears to be necessary for the uncoating of cores, since naked viral DNA is not liberated if it is prevented or impossible (as, for example, if cells are infected with heat- or urea-inactivated virus particles). It seems that an "uncoating protein" is synthesized that is capable of effecting the uncoating not only of the cores that code for it (homologous cores), but also heterologous ones. For example, if cells infected with vaccinia virus are superinfected some time (for example, three hours) later with particles of another poxvirus strain, they are immediately uncoated by the mediation of the uncoating protein specified by the first-infecting virus.

Once the viral DNA has been uncoated, it continues to be transcribed, apparently to roughly the same extent as DNA in cores. Relatively few of the proteins that are translated from this early messenger RNA are known. Some are enzymes such as a DNA polymerase, a thymidine kinase, a polynucleotide 5'-triphosphatase, and the capping enzymes. Some of the others, but only a very few, are capsid proteins, and some are proteins that become associated with the DNA just before and while it replicates. Vaccinia virus DNA replicates within a relatively brief interval of time, from about 2 to 4 hours after infection; vaccinia virus is atypical in this respect, since the DNAs of most other DNA-containing viruses replicate during the entire late period of the infection cycle.

Progeny DNA molecules are transcribed more extensively than parental ones: not only are some of the early sequences still transcribed from them, but also all the late ones. In fact, the vaccinia transcription pattern is extremely complex: it constantly changes, as is readily shown by pulse-labeling cells at hourly intervals and examining autoradiograms of polyacrylamide gels in which extracts of infected cells had been electrophoresed. This relatively simple technique is highly effective because vaccinia virus, like numerous other lytic viruses including reovirus, inhibits host protein synthesis very efficiently, so that even as early as one hour after infection most of the proteins formed in infected cells are virus-specified. When such gel autoradiograms are examined during the first 20 hours of the infection cycle (Fig. 4), it is clearly apparent that the pattern of viral gene expression constantly changes. Not only are there early proteins that are synthesized only during the early period, early proteins that continue to be synthesized in the late period, late proteins that are only synthesized in the early part of the late period and late proteins that are synthesized throughout the late period, but each of these four protein classes can be subdivided further; it may well be that most vaccinia proteins are under their own transcriptional and perhaps also translational controls [40,41]. In fact, the vaccinia virus multiplication cycle exhibits one of the relatively few well-known examples of translational control: it is known that the reason why the synthesis of early proteins such as the DNA polymerase or the thymidine kinase is arrested at the beginning of the late period is not the fact that their messenger RNAs are no longer synthesized and that their messenger RNAs are short-lived, but rather that their translation is actively prevented by a protein that is formed at the beginning of the late period. This phenomenon is known as the "switch-off" phenomenon [42]. It is demonstrated most readily by comparing the kinetics of early enzyme synthesis in cells infected with infectious vaccinia virus, with UV-inactivated vaccinia virus, and with both: in the first case early enzyme synthesis is switched off; in the second it is not; in the third it is [42]. Synthesis of early enzymes is therefore controlled not at the transcriptional, but at the translational level. Most of the remainder of the vaccinia virus gene expression program, however, is controlled at the transcriptional level.

Vaccinia virus morphogenesis proceeds as soon as progeny DNA molecules have

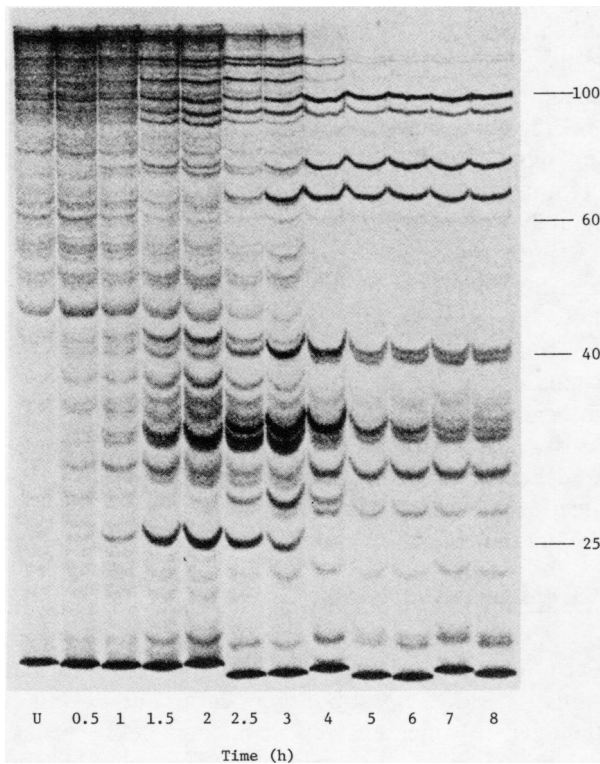


FIG. 4. The changing pattern of vaccinia virus specified polypeptide formation during the multiplication cycle. At the times indicated cells infected with vaccinia virus were labeled with  $^{35}\text{S}$ -methionine for one hour. The cells were then harvested, cytoplasmic cell extracts were prepared, and the polypeptides in them were separated by electrophoresis in SDS-polyacrylamide gels, which were then autoradiographed. U, uninfected cells; 0.5, etc., shows the polypeptide species synthesized during the time intervals from 0.5 to 1.5 h, etc., after infection. Note that the pattern of polypeptide synthesis changes throughout the multiplication cycle [38].

formed. Progeny DNA molecules are withdrawn at random from the pool of replicated DNA that accumulates between 2 and 4 h after infection and are then encapsidated via a series of reactions that, for each individual DNA molecule, take about one hour [43]. Obviously there must be numerous intermediates during the maturation of particles as complex as vaccinia virus particles, but few have been characterized. One that has been observed is a spherical particle that is composed of only two major polypeptide species, one being the core surface component, the other a phosphorylated polypeptide that is present in virions in very small amounts only [44-46].

#### *Comparison of the Multiplication Cycles of Reovirus and Vaccinia Virus*

It is seen that although the genomes of both reovirus and vaccinia virus consist of double-stranded nucleic acid, their gene expression strategies are quite different. In fact, the gene expression strategy of reovirus resembles much more closely that of the ss RNA containing viruses: reovirus can really be regarded as a virus whose genome is single-stranded RNA (which clearly provides the genetic continuity between parental and progeny virus particles), but which has the idiosyncrasy that the RNA form that is encapsidated is not ss (plus-stranded or minus-stranded) RNA, but its replicative form.

#### *The Identification of Type-Specific Poxvirus Proteins*

Smallpox virus appears to have been eliminated as a human pathogen; no naturally occurring cases of smallpox, either variola major or variola minor, have been reported during the last year, and WHO plans to pronounce smallpox

eradicated at the end of this year. This brings up the question of whether smallpox vaccination, which is still being carried out in Africa and Asia, should continue or whether it should cease. The decision is predicated largely on the potential effect upon man of several closely related viruses that occur or may occur in nature. These are of two types. The first is monkeypox virus, which causes a severe disease not only in monkeys but also in man, with a case mortality of up to 30 percent, but which is not transmissible, or only very poorly transmissible, from man to man; no epidemic of monkeypox virus in man has ever been observed, and even contacts of monkeypox virus cases do not contract the disease. The second are the so-called whitepox viruses which have been isolated both from monkey and from rodent tissues in various parts of the world, and which, according to all available evidence, are indistinguishable from smallpox virus. These whitepox viruses are either smallpox virus variants with an altered host range (since classical smallpox virus has only one host, man), in which case they pose a severe threat to man; or they are laboratory contaminants (since they were isolated in laboratories that were also handling smallpox virus at the time).

However that may be, it is clearly very important to devise reagents that can rapidly identify and differentiate between smallpox virus, whitepox viruses, and monkeypox virus. The best reagents for this purpose would be antibodies to type-specific poxvirus determinants. We are endeavoring to obtain such reagents in the following manner. First, we are devising techniques for identifying type-specific poxvirus determinants. This is being done by immunoprecipitating extracts of cells infected with various strains of poxvirus with antisera to both homologous and heterologous poxviruses, collecting the precipitates on staphylococcal A protein, and examining the virus-specific polypeptides in them by polyacrylamide gel electrophoresis followed by autoradiography. We have already shown that when homologous and heterologous immunoprecipitates of this type are compared, most viral polypeptides are precipitated by both homologous and heterologous antisera, but that some are only precipitated by homologous antisera. This is the case even when pairs of very closely related viruses are examined. The polypeptides that are only precipitated by homologous and not by heterologous antisera are clearly the type-specific ones.

As the next step we are attempting to isolate hybridomas to vaccinia virus-coded proteins, using for this purpose techniques that we have developed while preparing hybridomas to reovirus-coded polypeptides (we presently have hybridomas that produce monoclonal antibodies to seven of the ten reovirus proteins). Hybridomas that produce antibodies to type-specific poxvirus proteins will obviously be extremely useful for identifying any particular virus rapidly and definitively. Furthermore, knowledge of which poxvirus proteins are type-specific is also useful for screening animal sera for the presence of the corresponding antibodies, thereby providing evidence of previous exposure and infection.

In summary, I have compared the strategy of the multiplication cycles of reoviruses and poxviruses and described examples of how this information may be applied to problems relevant to diagnostic virology. There is every indication that the more is known about the molecular events that occur during virus multiplication, the more rapidly and definitively will one be able to detect and identify viral pathogens.

## REFERENCES

1. Shatkin AJ, Sipe JD, Loh PC: Separation of ten reovirus genome segments by polyacrylamide gel electrophoresis. *J Virol* 2:986-998, 1968
2. Smith RE, Zweerink HJ, Joklik WK: Polypeptide components of virions, top component and cores of reovirus type 3. *Virology* 39:791-810, 1969

3. Joklik WK: Reproduction of reoviridae. Comprehensive Virology, Vol 2. Edited by H Fraenkel-Conrat and RR Wagner. New York and London, Plenum Press, 1974, pp 231-334
4. Zweerink HJ, Joklik WK: Studies on the intracellular synthesis of reovirus-specified proteins. Virology 41:501-518, 1970
5. Shatkin AJ, Sipe JD: RNA polymerase activity in purified reoviruses. Proc Natl Acad Sci USA 61:1462-1469, 1968
6. Borsa J, Graham AF: Reovirus: RNA polymerase activity in purified virions. Biochem Biophys Res Commun 33:896-901, 1968
7. Skehel JJ, Joklik WK: Studies on the *in vitro* transcription of reovirus RNA catalyzed by reovirus cores. Virology 39:822-831, 1969
8. Joklik WK: Studies on the effects of chymotrypsin on reovirions. Virology 49:700-715, 1972
9. Luftig RB, Kilham SS, Hay AJ, et al: An ultrastructural study of virions and cores of reovirus type 3. Virology 48:170-181, 1972
10. McCrae MA, Joklik WK: The nature of the polypeptide encoded by each of the ten double-stranded RNA segments of reovirus type 3. Virology 89:578-593, 1978
11. Mustoe TA, Ramig RF, Sharpe AH, et al: Genetics of reovirus: Identification of the ds RNA segments encoding the polypeptides of the  $\mu$  and  $\sigma$  size classes. Virology 89:594-604, 1978
12. Nichols JL, Bellamy AR, Joklik WK: Identification of the nucleotide sequences of the oligonucleotides present in reovirions. Virology 49:562-572, 1972
13. Silverstein SC, Astell C, Christman J, et al: Synthesis of reovirus oligo adenylic acid *in vitro* and *in vivo*. J Virol 13:740-752, 1974
14. Johnson RB Jr, Soeiro R, Fields BN: The synthesis of A-rich RNA by temperature-sensitive mutants of reovirus. Virology 73:173-180, 1976
15. Chang C, Zweerink HJ: Fate of parental reovirus in infected cell. Virology 46:544-555, 1971
16. Silverstein SC, Astell C, Levin DH, et al: The mechanisms of reovirus uncoating and gene activation *in vivo*. Virology 47:797-806, 1972
17. Shatkin AJ: Methylated messenger RNA synthesis *in vitro* by purified reovirus. Proc Natl Acad Sci USA 71:3204-3207, 1974
18. Desrosiers RC, Sen GC, Lengyel P: Difference in 5' terminal structure between the mRNA and the double-stranded virion RNA of reovirus. Biochem Biophys Res Commun 73:32-39, 1976
19. Nichols JL, Hay AJ, Joklik WK: 5' terminal nucleotide sequence of reovirus mRNA synthesized *in vitro*. Nature New Biology 235:105-107, 1972
20. Kozak M, Shatkin AJ: Identification of features in 5' terminal fragments from reovirus mRNA which are important for ribosome binding. Cell 13:201-212, 1978
21. Lazarowitz SG, Robertson HD: Initiator regions from the small size class of reovirus messenger RNA protected by rabbit reticulocyte ribosomes. J Biol Chem 252:7842-7849, 1977
22. Acs G, Klett H, Schomberg M, et al: Mechanism of reovirus double-stranded ribonucleic acid synthesis *in vivo* and *in vitro*. J Virol 8:684-689, 1971
23. Sakuma S, Watanabe Y: Incorporation of *in vitro* synthesized reovirus double-stranded ribonucleic acid into reovirus corelike particles. J Virol 10:943-950, 1972
24. Morgan EM, Zweerink HJ: Characterization of transcriptase and replicase particles isolated from reovirus-infected cells. Virology 68:455-466, 1975
25. Morgan EM, Zweerink HJ: Reovirus morphogenesis: corelike particles in cells infected at 39° with wild-type reovirus and temperature-sensitive mutants of groups B and G. Virology 59:556-565, 1974
26. Lai MT, Joklik WK: The induction of interferon by temperature-sensitive mutants of reovirus, UV-irradiated reovirus, and subviral reovirus particles. Virology 51:191-204, 1973
27. Martinson HG, Lewandowski LJ: Sequence homology studies between the double-stranded RNA genomes of cytoplasmic polyhedrosis virus, wound tumor virus and reovirus strains 1, 2 and 3. Intervirology 4: 91-98, 1974
28. Gaillard RK, Joklik WK: Unpublished results
29. Weiner HL, Fields BN: Neutralization of reovirus: the gene responsible for the neutralization antigen. J Exp Med 146:1305-1310, 1977
30. Weiner HL, Ramig RF, Mustoe TA, et al: Identification of the gene coding for the hemagglutinin of reovirus. Virology 86:581-584, 1978
31. Weiner HL, Drayna D, Averill DR Jr, et al: Molecular basis of reovirus virulence: the role of the S1 gene. Proc Nat Acad Sci USA 74:5744-5748, 1977
32. Finberg R, Weiner HL, Fields BN, et al: Generation of cytolytic T lymphocytes after reovirus infection: Role of S1 gene. Proc Natl Acad Sci USA 76:442-446, 1979
33. Wittek R, Menna A, Schümperli D, et al: Hind III and Sst I restriction sites mapped on rabbitpox virus and vaccinia virus DNA. J Virol 23:669-687, 1977

34. Wittek R, Menna A, Mueller HK, et al: Inverted terminal repeats in rabbitpox virus and vaccinia virus DNA. *J Virol* 28:171-181, 1978
35. Garon CF, Barbosa E, Moss B: Visualization of an inverted terminal repetition in vaccinia virus DNA. *Proc Natl Acad Sci USA* 75:4863-4867, 1978
36. Sanger F, Air GM, Barrell BG, et al: Nucleotide sequence of bacteriophage  $\phi$ X 174 DNA. *Nature* 265:687-695, 1977
37. Smart JE, Ito Y: Three species of polyoma virus tumor antigens share common peptides probably near the amino termini of the proteins. *Cell* 15:1427-1437, 1978
38. Paoletti E: In vitro synthesis of a high molecular weight virion-associated RNA by vaccinia. *J Biol Chem* 252:866-871, 1977
39. Pelham HRB: Use of coupled transcription and translation to study mRNA production by vaccinia cores. *Nature* 269:532-534, 1977
40. Cooper JA, Moss B: Transcription of vaccinia virus mRNA coupled to translation *in vitro*. *Virology* 88:149-165, 1978
41. Pennington TH: Vaccinia virus polypeptide synthesis: sequential appearance and stability of pre- and post-replicative polypeptides. *J Gen Virol* 25:433-444, 1974
42. McAuslan BR: Control of induced thymidine kinase activity in the poxvirus-infected cell. *Virology* 20:162-168, 1963
43. Joklik WK, Becker Y: The replication and coating of vaccinia DNA. *J Mol Biol* 10:452-474, 1964
44. Sarov I, Joklik WK: Isolation and characterization of intermediates in vaccinia virus morphogenesis. *Virology* 52:223-233, 1973
45. Morgan C: Vaccinia virus reexamined: development and release. *Virology* 73:43-58, 1976
46. Nagayama A, Pogo BGT, Dales S: Biogenesis of vaccinia: separation of early stages from maturation by means of rifampicin. *Virology* 40:1039-1051, 1970