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Molecular Mechanisms of Virus-Mediated Cytopathology [and Discussion]

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Molecular mechanisms of virus-mediated cytopathology

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Lytic virus infections of animal cells usually lead to a variety of morphological and biochemical lesions that include inhibition of cellular macromolecular syntheses. These cytopathic effects vary in intensity for different virus–cell combinations and probably involve several overlapping mechanisms. Inhibition may be mediated by components of parental virions or require viral gene expression. In many infected cell systems the initiation of host protein synthesis is selectively blocked. This shut-off phenomenon can result from changes in membrane permeability that alter the intracellular ionic environment in favour of viral expression, successful competition of viral mRNAs for limited translational components, or a decrease in the level of cell mRNAs by inhibition of synthesis or nucleocytoplasmic transport. However, the early onset and rapidity of virus-induced inhibition, sometimes under non-permissive conditions, implies more direct mechanisms of translational inactivation. These include enhanced degradation of cellular mRNAs or specific modification of the translation apparatus in infected cells. A dramatic example of the latter occurs in poliovirus-infected HeLa cells in which intact, functional cellular mRNA persists but host protein synthesis is almost completely inhibited. The virus-induced defect is apparently related to inactivation of a protein factor that binds to the 5′ end of m^7G-capped mRNAs and is required for translation of host (capped) mRNAs but not for the expression of poliovirus RNA, which is not capped. This process and other possible molecular mechanisms of virus-mediated cytopathology are discussed.

Overview of inhibitory effects of animal viruses

A review of how DNA and RNA viruses redirect and ultimately kill infected cells provides no obvious or simple unifying theme. As predicted from the multifarious nature of animal viruses and host cell types, the mechanisms of virus-mediated cytopathology are many and diverse. This variety is evident from consideration of a single property of most lytic viruses, the capacity to inhibit, often dramatically, the initiation of cellular protein synthesis in favour of virus-specific polypeptide formation.

The molecular basis of selective inhibition is not well understood. However, it seems clear that even closely related viruses have evolved distinct schemes to accomplish it. For example, in Friend mouse erythroleukaemia cells, herpes simplex virus type 1 (HSV-1) shuts off host protein synthesis gradually and only after viral gene expression. In the same cell line, type 2 herpes simplex virus inhibits rapidly, apparently by direct action of a virion-associated component (Hill *et al.* 1983; see also Fenwick & Walker 1978). Among RNA viruses, two similar picornaviruses, poliomyelitis and encephalomyocarditis virus (EMC), inhibit host protein synthesis in HeLa cells by different mechanisms (Jen *et al.* 1980).

Cell type has a correspondingly important influence on the nature of the cytopathic response to infection by a particular virus. One of the first and most striking examples of this was observed with the simian paramyxovirus SV5. In primary monkey kidney cells SV5 replication is accompanied by minimal effects on host processes, whereas in hamster cells the virus is cytocidal

(Holmes & Choppin 1966). Protein synthesis is markedly inhibited in mouse L cells infected with reovirus type 3 (Skup & Millward 1980; Detjen *et al.* 1982) or EMC virus (Jen & Thach 1982). In another mouse fibroblast line (SC-1), infection by the same viruses appears not to alter the translational machinery directly, and cell protein synthesis declines at later times after infection (Detjen *et al.* 1982; Jen & Thach 1982).

Despite the complexity of cytopathogenesis, several well studied systems illustrate some of the mechanisms that viruses use to overtake a functioning cellular metabolism. In poliovirus-infected HeLa cells one (or more) of the initiation factors is modified, and consequently viral mRNAs are translated almost exclusively (Helentjaris & Ehrenfeld 1978). Potentially functional host mRNAs remain intact but incapable of forming initiation complexes in infected cells (Leibowitz & Penman 1971).

In striking contrast, cellular mRNAs are degraded in fowl plague virus-infected chick embryo cells (Inglis 1982) and in hamster and mouse cells infected with HSV-1 (Inglis 1982; Nishioka & Silverstein 1978). Viral mRNAs also become predominant during adenovirus replicaton in HeLa cells but in this system by selective nucleocytoplasmic transport of viral transcripts (Beltz & Flint 1979). In both situations the resulting excess of viral mRNAs presumably facilitates access to a limited number of ribosomes and preferential synthesis of viral polypeptides without alteration of host translational components.

In addition to diverting transcriptional and translational events, viruses can directly or indirectly disrupt cell structure. Adsorption of virus particles to cell surface receptors increases lipid fluidity of the plasma membrane (Levanon *et al.* 1977), and insertion of nascent virus-coded proteins into intracellular and surface membranes changes many parameters including permeability to ions and other substances (Contreras & Carrasco 1979). A general hypothesis to explain inhibition of host translation is based on virus-induced membrane leakiness and increased intracellular sodium ions (Carrasco 1977). Since many viral but few cellular mRNAs can initiate translation at elevated Na^+ concentrations (Nuss *et al.* 1975), a shift from host to viral products would follow a loss in the capacity of the cell membrane to maintain a Na^+/K^+ gradient. Results compatible with this model have been reported for a number of virus-cell systems (Carrasco & Smith 1980), notably Sindbis virus-infected chick cells (Garry *et al.* 1979). On the other hand, recent studies of vaccinia virus-infected HeLa cells demonstrate that the switch from host to viral protein synthesis is essentially complete within 4 h after infection, whereas the average intracellular Na^+/K^+ ratio is reversed only several hours later (Norrie *et al.* 1982; see also Bossart & Bienz 1981).

These different examples illustrate the multiplicity of virus-mediated cytopathic effects and point out the difficulties of trying to explain them by one general mechanism. A more detailed consideration of individual viruses reveals that cytopathogenesis may be induced by a combination of superimposed mechanisms including inhibition of the host 'from without' by the direct action of parental particles and 'from within' by virus-induced products.

Vaccinia, a highly cytocidal DNA virus

Infection with vaccinia or other poxviruses causes characteristic morphological and biochemical lesions. At high multiplicity of infection, cells usually become rounded, synthesis of cell DNA, RNA and protein decreases, and structural degeneration ensues (Bablanian 1975; Moss 1974). Considerable effort has been made to determine if these events require viral gene

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expression or are mediated by infecting virions. Initial studies favoured the idea that a virion-associated cytotoxic component causes early cell rounding because it occurred in the presence of inhibitors of replication and was more rapid at higher multiplicities. However, it was shown subsequently that inhibitors of protein synthesis prevent early cell rounding, indicating that the effect requires formation of virus-induced polypeptide(s) and may not be caused directly by a virion component (Bablanian 1975). The exact nature of the inducer of cell rounding, whether it is coded for by host or viral genes, and its mechanism of action remain to be established.

One of the first biochemical lesions detectable after vaccinia virus infection is a striking decrease in host mRNA translation. At high multiplicities of infection, inhibition is almost complete within 30 min in the presence of actinomycin (Moss 1968). The inhibition is apparently not due to degradation of cellular templates because mRNAs isolated from infected and uninfected cells are translated equally well in reticulocyte lysate (Cooper & Moss 1979). In contrast to protein synthesis, host RNA synthesis may continue early in infection, but transport from the nucleus to the cytoplasm is decreased (Salzman *et al.* 1964). Because vaccinia virus replicates in the cytoplasm, viral transcripts may compose the bulk of the newly made cytoplasmic RNAs as cellular nucleic acid synthesis declines. As described below, transcripts produced by the virion-associated polymerase may play a central role in host cell inhibition by vaccinia virus.

Evidence has been obtained in support of at least two distinct but probably overlapping mechanisms for poxvirus-induced cessation of host protein synthesis. In one scheme, structural polypeptides released from virions during the very early stages of infection directly alter ribosomes or other translation component(s), resulting in a block in the initiation of protein synthesis shortly after infection. Although vaccinia surface tubule (s.t.) protein isolated from virions or infected cells was cytotoxic when introduced into cells by hypertonic shock, it did not rapidly inhibit protein synthesis by intact HeLa cells or cell-free extracts (Wolstenholme *et al.* 1977; Burgoyne & Stephen 1979). These results indicated that this viral cytotoxin acts from within, possibly causing the degeneration of cells that occurs at late times after vaccinia infection, rather than from without at the time of the early shut-off phenomenon. By contrast, Mbuy *et al.* (1982) have suggested that s.t. protein is a selective inhibitor of translation. Exposure of human HEp-2 cells to high levels of s.t. protein (equivalent to 10^4 particles per cell) resulted in a decreased number of polyribosomes and a marked inhibition in the rate of incorporation of amino acids (but not thymidine or uridine) into acid-precipitable material. Translation of exogenous globin mRNA in reticulocyte lysate was also inhibited by the viral protein; vaccinia-specific mRNAs were not tested. The correspondence between inhibitor and s.t. protein was indicated by specific antibody tests. However, the possibility that other inhibitory components co-purified with the s.t. protein was not excluded.

Consistent with the proposal that component(s) of parental virions are responsible for host shut-off, binding of initiator tRNA to 40*S* ribosomal subunits was decreased in extracts prepared from vaccinia-infected mouse ascites cells that had been pretreated with cordycepin to prevent viral transcription (Person *et al.* 1980). Similarly, 40*S* Met-tRNA$_i^{Met}$ complex formation was inhibited in rabbit reticulocyte lysate by purified vaccinia cores. However, because mRNA binding to 40*S* ribosomal subunits occurs afterwards, and is dependent on prior attachment of initiator tRNA, inhibition at this step of initiation would adversely affect the template activity of viral as well as host messengers. Indeed, the presence of vaccinia cores

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diminished translation in reticulocyte lysate non-selectively, decreasing the activity of vaccinia-specific early mRNAs as well as several exogenous mRNAs including globin mRNA (Person & Beaud 1980). This suggests that viral cores may inhibit non-specifically in cell extracts, a possibility supported by the finding that vaccinia cores inhibit translation of plant virus RNA in wheat germ extract (Ben-Hamida & Beaud 1978).

Although the results *in vitro* do not account for selective inhibition of the host, discrimination in vaccinia-infected cells may be due to a complete block in protein synthesis followed by selective reversal by a very early viral product (Moss & Filler 1970). If such a discriminatory element is involved, it has not been identified. Nor has the initiation inhibitory component in cores, although it has been dissociated from cores recently (Ben-Hamida *et al.* 1983). The solubilized and partly purified core fraction was micrococcal nuclease-resistant and included basic phosphoproteins of apparent molecular masses 9–15 kDa (Ben-Hamida *et al.* 1983) but perhaps fragments of s.t. protein as well (Mbuy *et al.* 1982). Met-tRNA$_i^{Met}$ binding to reticulocyte 40*S* ribosomes was inhibited by the soluble preparation. It will be of interest to explore the effect of purified inhibitor on ribosomes and soluble components of cell-free initiating systems and to determine if there are any reversible or cell mRNA-specific modifications.

Another mechanism for vaccinia virus-mediated shut-off of host translation depends upon viral gene expression, specifically the formation of inhibitory transcripts by the virion-associated enzyme complex. Early experiments showed that shut-off proceeded in infected HeLa cells treated with actinomycin (2 μg ml^{-1}), implying direct inhibition (Shatkin 1963). Subsequently this drug concentration was found to be inadequate to block virion expression completely (Rosemond-Hornbeak & Moss 1975). Short RNA products were formed in the presence of the inhibitor. Furthermore, these poly(A)-rich RNAs were associated with small, inactive polyribosomes, possibly preventing host mRNA attachment. Short transcripts were also produced by u.v.-irradiated vaccinia virus both *in vitro* and in infected HeLa cells. Heavily irradiated, replication-defective particles that retained some transcriptase activity were capable of blocking host protein synthesis. Concentrations of actinomycin (20–30 μg ml^{-1}) presumably sufficient to inhibit all RNA synthesis prevented virus-induced shut-off (Bablanian *et al.* 1981).

Correlation of low molecular mass viral RNA synthesis and inhibition of host translation under a variety of experimental conditions suggests a causal relation (Coppola & Bablanian 1983). Vaccinia virus *in vitro* transcripts inhibited the translation of globin mRNA, EMC virus RNA, and total cytoplasmic RNAs from HeLa and hamster cells in reticulocyte lysate. The inhibitory effect, like that in vaccinia virus-infected cells, was selective; virion transcripts did not diminish the pattern of early viral polypeptide products in lysate directed by RNA extracted from HeLa cells at 2½ h after infection. Low but inhibitory levels of *in vitro* transcripts had no detectable template activity, suggesting inhibition by the RNA rather than via a nascent polypeptide intermediate. RNA extracted from HeLa cells at early times after vaccinia virus infection also inhibited cell-free translation of globin mRNA. More importantly, virus-specific RNA isolated from purified viral cores selectively decreased the translation of exogenous cellular RNAs in reticulocyte lysate. These findings may account for some of the direct inhibitory effects of viral cores on initiation of translation (Person *et al.* 1980). If this proves true, it would reconcile two apparently disparate explanations for the vaccinia virus-mediated shut-off of host functions, not an unusual turn of events in research.

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Translational control in poliovirus-infected HeLa cells: influence of mRNA 5′-terminal structure

Much effort has been made to elucidate the molecular basis of a finding reported two decades ago, that in poliovirus-infected HeLa cells host polyribosomes are rapidly disaggregated and replaced by the corresponding virus-specific structures (Penman *et al.* 1963; Ehrenfeld 1982). As a consequence of this change, HeLa cells are effectively reprogrammed for the exclusive production of viral polypeptides shortly after poliovirus infection. Host shut-off also takes place under conditions that prevent virus replication. Several lines of evidence indicate that the inhibition is mediated by protein(s) coded for by input virion RNA, possibly augmented at high multiplicities of infection by parental capsid protein (Holland 1964):

(i) shut-off proceeded in the presence of guanidine, a potent inhibitor of virus RNA replication (Bablanian *et al.* 1965; Penman & Summers 1965; Helentjaris & Ehrenfeld 1977) that also blocks Na^+ accumulation (Nair *et al.* 1979) and delays the onset of morphological changes in poliovirus-infected human cells (Bablanian *et al.* 1965);

(ii) host translation in cells infected in the presence of puromycin or cycloheximide was inhibited after the removal of the drug but only after a period of viral protein synthesis (Penman & Summers 1965);

(iii) studies with u.v.-inactivated virions indicated that a functional viral genome is imperative for host shut-off (Penman & Summers 1965; Helentjaris & Ehrenfeld 1977);

(iv) viral mutants defective in structural protein failed to shut off the host at the restrictive temperature (Steiner-Pryor & Cooper 1973).

Although the viral protein putatively responsible for host shut-off has not been identified, the target of inactivation has been localized to the protein synthesis initiation factors. Ribosomal subunits and salt-washed polyribosomes obtained from poliovirus-infected cells were active for translation initiation *in vitro* (Kaufmann *et al.* 1976), and cellular mRNAs were not structurally or functionally damaged after infection (Fernandez-Munoz & Darnell 1976). However, ribosomal salt wash from infected cells contained initiation factors with diminished activity for cellular mRNA translation (Kaufmann *et al.* 1976). Helentjaris & Ehrenfeld (1978) pursued this observation and showed that crude initiation factors from infected cells were strikingly altered. Unlike uninfected cell factors that were capable of stimulating initiation with either viral or host mRNAs, infected cell factors were active only with poliovirus-specific polyribosomes. Modification of eukaryotic initiation factor 2 (eIF-2) by poliovirus infection was ruled out because the formation of a ternary complex with GTP and initiator tRNA was similar with factors from both infected and uninfected cells. It was reasonable then to focus on the subsequent steps of initiation, specifically on initiation factors that promote mRNA binding to 40*S* ribosomal subunits.

Initiation factors fractionated from uninfected and poliovirus-infected HeLa cells were compared in an attempt to identify the basis for mRNA discriminatory activity. An infected cell fraction that included eIF-4B and eIF-3, initiation factors involved in mRNA attachment to ribosomal subunits (for review see Maitra *et al.* 1982), stimulated translation of poliovirus RNA but not cell mRNA. The corresponding uninfected cell fraction was active with both types of messenger (Helentjaris *et al.* 1979). On the basis of these and additional studies it was suggested that eIF-3, or a factor that co-purifies with it, is the altered component responsible for selective initiation of viral RNA translation in poliovirus-infected cells.

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By contrast, Rose *et al.* (1978) concluded from *in vitro* translation studies that inactivation of eIF-4B was probably the mechanism of poliovirus-induced host shut-off. These apparently contradictory results were at least partly resolved by the finding that partially purified eIF-3 and eIF-4B both contain a *ca.* 24 kDa 'cap-binding' polypeptide that can be separated in buffers of high ionic strength (Sonenberg *et al.* 1978; Trachsel *et al.* 1980). The *ca.* 24 kDa protein binds to the 5′ end of m^7GpppN-terminated (capped) mRNAs as detected by specific cross-linking to oxidized mRNA (Sonenberg *et al.* 1978). Most cellular and viral mRNAs of eukaryotes contain a cap, and the presence of this structure facilitates initiation of translation (Shatkin 1976). Cap-binding protein differentially stimulated translation of capped mRNA in extracts derived from uninfected HeLa cells (Sonenberg *et al.* 1980). This positive functional effect fits an overall scheme of initiation in which ribosomes bind at the 5′ end of mRNA and (usually) begin polypeptide synthesis at the cap-proximal A-U-G codon (Kozak 1981). The association of cap-binding protein with other initiation factors may also have functional significance: for example, eIF-3 on native 40*S* ribosomal subunits may contribute to the stabilization of initiation complexes by interacting with cap-binding protein attached to incoming mRNA. Poliovirus RNA (like other picornavirus RNAs) is a notable exception among eukaryotic messengers because it does not contain a 5′ cap (Nomoto *et al.* 1976; Hewlett *et al.* 1976). Its translation is apparently cap-independent. Thus the inactivation of cap-binding protein in poliovirus-infected HeLa cells may account for the accompanying translational switch from host (capped) mRNA to viral (uncapped) RNA. The same model may apply to reovirus-infected mouse L cells because cell-free extracts prepared at late times after infection preferentially translated uncapped viral mRNAs (Skup & Millward 1980).

This mechanism of poliovirus-mediated host shut-off gained support when it was demonstrated that the translation of capped mRNAs is restored in extracts of poliovirus-infected HeLa cells by the addition of cap-binding protein isolated from rabbit reticulocyte ribosomal salt wash (Trachsel *et al.* 1980). 'Restoring factor' co-purified with the 24 kDa cap-binding protein, but the most highly purified activity was labile, suggesting the involvement of additional polypeptides. Re-examination of the nature of stable restoring activity showed that it corresponds to a large, *ca.* 8*S* protein complex consisting of the 24 kDa cap-binding protein and at least two other components: a *ca.* 220 kDa polypeptide and initiation factor eIF-4A (*ca.* 46 kDa), which also cross-links to the 5′ end of oxidized, capped mRNA (Tahara *et al.* 1981, 1982; Grifo *et al.* 1982). Restoring activity was also obtained in a fast-sedimenting complex from uninfected but not from poliovirus-infected HeLa cells (Tahara *et al.* 1981). The modification(s) responsible for host shut-off and inactivation of restoring factor have been separately attributed to dissociation of the 24 kDa cap-binding protein from a high molecular mass functional complex (Hansen *et al.* 1982), loss of cap-binding activity of the 24 kDa (and other) polypeptide(s) by the action of a component in infected cell initiation factors (Lee & Sonenberg 1982) and proteolysis of the *ca.* 220 kDa component of the cap-binding protein complex (Etchison *et al.* 1982). Whether these effects are primary or causal events, or both, in the shut-off process in poliovirus-infected HeLa cells remains to be determined.

Multiple mechanisms of cytopathogenesis by RNA viruses

Intriguing variations on the theme of virus-induced inhibition of cell protein synthesis have been described. For example, at late times after infection with vesicular stomatitis virus (VSV),

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only viral proteins are produced. As in poliovirus-infected cells, host mRNA remains and can be extracted and translated *in vitro* (Ehrenfeld & Lund 1977). It seems clear that the block does not depend upon differences in mRNA 5′-terminal structure because VSV mRNAs are capped and apparently require cap-binding protein for translation (Banerjee 1980; Rose *et al.* 1978). VSV-induced shut-off probably involves mRNA competition, initiation factor eIF-2 alteration and inhibition by viral transcripts by mechanisms that are being actively explored in several laboratories (Lodish & Porter 1981; Centrella & Lucas-Lenard 1982; Schnitzlein *et al.* 1983; Dunigan & Lucas-Lenard 1983).

In Semliki Forest virus-infected mouse cells, cap-binding activity is decreased, and host translation is concomitantly and selectively inhibited (van Steeg *et al.* 1981). However, the two virus-specified messengers are both capped (Pettersson *et al.* 1980). Translation of the predominant late 26*S* mRNA that encodes viral capsid protein is relatively cap-independent (van Steeg *et al.* 1981). Consequently, early formation of viral replicase directed by virion 42*S* RNA may precede modification of translation factors that result in selective synthesis of viral capsid protein at later times after infection.

Cells infected with two different viruses can also provide information about specific inhibitory events related to cytopathogenesis. For example, expression of SV5 mRNA and late 26*S* Sindbis mRNA (both capped) continues in poliovirus superinfected cells under conditions in which cellular protein synthesis and VSV mRNA translation are inhibited (Choppin & Holmes 1967; Alonso & Carrasco 1982). In each case, host initiation factors are presumably modified (Brown *et al.* 1980). Variable levels of dependence on cap-binding protein and other factors may explain these differences.

Concluding remarks

A general impression derived from a consideration of representative animal viruses is that virus-mediated cytopathology is a weave of complex processes that are only beginning to be unravelled at the molecular level. Some elements of cytopathogenesis discovered by studying cells infected with vaccinia or poliovirus are likely to apply to other systems. However, these will vary both qualitatively and quantitatively as a function of the host cell and the infecting particle. Much remains to be learned.

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Discussion

C. A. Pasternak (*Department of Biochemistry, St George's Hospital Medical School, London, U.K.*). Several lines of evidence point to the possibility that certain viruses may have a 'toxin-like' action analogous to that of bacterial toxins. Haemolytic paramyxoviruses provide a good example.

U.v.-inactivated, non-infectious Sendai virus or Newcastle disease virus elicits a Ca^{2+}-sensitive permeability change in the plasma membrane of susceptible cells that has the following characteristics (Pasternak & Micklem 1981): ions and compounds of low molecular mass leak into, and out of, cells down ther respective concentration gradients. Unlike the situation with frog virus 3 (Kirn & Keller, this symposium), these viruses are not cytolytic and compounds of high molecular mass do not leak out of cells; they are haemolytic only because the colloid osmotic swelling that results from the changes in monovalent cations cannot be tolerated by erythrocytes. The molecular basis for the permeability change appears to involve the fusion of potentially leaky, viral envelope components with the cell plasma membrane. Because such virally derived 'channels' are subject to dispersal by lateral diffusion, as well as to removal by membrane 'recycling', it it not surprising that the permeability change is transient. Cells whose physiological functions are affected by plasma membrane depolarization or Ca^{2+} entry, or both (both of which are consequences of the virally induced permeability change), thus react to the presence of virus in a reversible manner: the electrical conductivity of cultured neurons, the beating of cultured heart cells and the secretion of hormones by anterior pituitary cells (Forda *et al.* 1982) are transiently altered in otherwise intact cells by non-infectious virus.

The relevance of such alterations in cellular function to the possible role of leucocytes in mediating the effects of viral infections is currently under study.

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