Interferon Induction by Viruses. III. Vesicular Stomatitis Virus: Interferon-inducing Particle Activity Requires Partial Transcription of Gene N

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SUMMARY

We have measured the interferon-inducing particle (i.f.p.) activity of a ts mutant, G11 (I), of vesicular stomatitis virus (VSV) and a non-ts revertant, R1 (T1026) in 'aged' chick embryo cells and mouse L(Y) cells at 40.5 and 37.5°C, respectively. Our results suggest that a single i.f.p. suffices to induce a quantum yield of interferon and that there are several times more i.f.p. than plaque-forming particles (p.f.p.) in stock preparations of VSV. Furthermore, while virus replication or amplified RNA synthesis is not required for a particle of VSV to induce interferon, there is a requirement for primary transcription. About one-tenth of the genome must remain intact and be transcribed to synthesize an interferon-inducer moiety. (This represents transcription of about two-thirds of the N protein gene.)

We conclude that VSV does not contain a pre-formed inducer of interferon and propose a model for its formation. We suggest that there is a cumulative loss of N (and/or NS and L) protein from the ribonucleoprotein complex during primary transcription, leading ultimately to extensive base-pairing between the genome RNA and its complementary transcript. We suggest that the dsRNA thus formed constitutes the interferon inducer moiety of VSV.

INTRODUCTION

Vesicular stomatitis virus (VSV) is generally considered to be a poor inducer of interferon (Paucker et al. 1962; Wagner et al. 1963; Wagner & Huang, 1966). However, under certain conditions significant amounts of interferon can be induced by standard preparations, or mutants, of this virus, both in vivo (Youngner & Wertz, 1968) and in vitro (Wertz & Youngner, 1970; Ramseur & Friedman, 1977, 1978; Nishiyama et al. 1978; Francoeur et al. 1978; Youngner et al. 1978; Sekellick & Marcus, 1979a). The presence of interferon can be inferred from specific experimental manipulations of anti-interferon serum in vitro (Vengris et al. 1975; Vilcek et al. 1975; DeClercq, 1978; Sekellick & Marcus, 1978) – or cholera toxin (Kohn et al. 1976) – or in vivo (Gresser et al. 1976).

Recently, we have demonstrated that all VSV mutants which display a psi− (protein synthesis inhibition) and ckp− (cell killing particle) phenotype are excellent inducers of interferon at non- and semi-permissive temperatures, as are some mutants expressing a psi+, ckp+ phenotype (Sekellick & Marcus, 1979a). We have used two of these excellent inducers of interferon, the ts mutant G11 (I) and the non-ts revertant R1 of T1026(I) in the hyper-responsive aged chick embryo cell system (Carver & Marcus, 1967; Marcus & Sekellick, 1977) and in L-cells to determine what VSV gene functions are required for inter-
feron induction. In this communication we show that (i) a single interferon-inducing particle (i.f.p.) of VSV suffices to produce a quantum yield of interferon, (ii) neither virus replication nor amplified RNA synthesis is required for i.f.p. activity, (iii) standard preparations of VSV may contain several times more i.f.p. than plaque-forming particles (p.f.p.), (iv) i.f.p. require a functional transcriptase, which in turn, (v) must transcribe at least about one-tenth of the virus genome in order to synthesize an interferon-inducer moiety. Based on these observations, we suggest a model for the formation of the interferon inducer, presumably a molecule of dsRNA.

METHODS

Cells and medium. The preparation and in vitro 'ageing' of monolayers of primary chick embryo cells has been described (Carver & Marcus, 1967; Marcus & Sekellick, 1977). Cells cultured and 'aged' under our conditions produce large amounts of interferon upon appropriate stimulation with particulate inducers and are more responsive to the action of interferon (Carver & Marcus, 1967; Marcus & Sekellick, 1977; Marcus & Fuller, 1979). Maximal amounts of interferon are induced at 40-5 °C (Sekellick & Marcus, 1979a). The growth and use of mouse L(Y) cells in interferon induction studies have been described (Sekellick & Marcus, 1978). This line produced more interferon upon induction than five other lines of mouse cells (Sekellick & Marcus, 1979b). Maximal levels of interferon were induced in L(Y) cells at 37-5 °C.

Viruses: source and stock preparation. Conditions for the growth and purification of virus stocks of VSV (Indiana) have been described (Marcus & Sekellick, 1974). Mutant tsGI (I) is from Glasgow (Pringle, 1970) and for the purpose of this study can be defined phenotypically at 40-5 °C as tra+ (primary transcription), ifp+ (interferon-inducing particle), psi− (cellular protein synthesis inhibition), ckp− (cell-killing particle), rep− (RNA replication) and pfp− (plaque-forming particle) (Marcus & Sekellick, 1975; Marvaldi et al. 1977; Sekellick & Marcus, 1980). Revertant R1 is from Toronto and represents a non-ts revertant of tSTI026(I) (Stanners et al. 1977). All p.f.p. titres refer to assays carried out at 30 °C on Vero cells, themselves incapable of expressing the ifp+ phenotype (Desmyter et al. 1968; Sekellick & Marcus, 1978; Emeny & Morgan, 1979).

In all experiments we included a defective-interfering particle of VSV, DI-o11 as a standard i.f.p. with the presumed capacity to assume in cells a dsRNA configuration from pre-existing covalently linked self-complementary [± ]RNA (Lazzarini et al. 1975; Marcus & Sekellick, 1977).

Inactivation of virus by u.v. radiation. Virus stocks were inactivated with u.v. radiation as described previously (Marcus & Sekellick, 1975). The germicidal lamp delivered a flux of 64 ergs/mm²/10 s. The D°,σ,37 (1/e, 37% survival) = 52.3 ergs/mm².

Inactivation of virus by heat. Virus preparations were inactivated in a circulating water bath maintained at 50±1 °C. At the start, a 1:10 dilution of stock virus was made in NCI medium (Marcus & Carver, 1965) contained in a glass tube pre-warmed to 50 °C. Tubes were removed at appropriate intervals and chilled rapidly by placing them in a salted ice bath.

Interferon induction and assays. Details concerning the induction and assay of interferon have been described (Marcus & Sekellick, 1976; Sekellick & Marcus, 1979a; Marcus & Fuller, 1979). We wish to add that during the induction period serum was omitted from the medium because some batches had a deleterious effect on the yield of interferon (Sekellick & Marcus, 1979a). Induction on 'aged' chick cells and mouse L-cells was carried out at 40-5 and 37-5 °C, respectively.
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Fig. 1. Dose-response curve relating multiplicity of infection of VSV tsG11(I) to the interferon yield on 'aged' (6 days) chick embryo cells (CEC, 1st passage) at 40-5 °C. 

Fig. 2. Dose-response curve relating multiplicity of infection (p.f.p.) of VSV non-ts revertant R1 (Tio26) to the interferon yield on 'aged' chick embryo cells (1st passage) at 40-5 °C.

In our assay 1 PR50 (50% plaque reduction titre) (VSV) unit was equivalent to about 10 to 25 units, in terms of the research reference chick interferon standard 63/A, kindly supplied by the National Institutes of Health Resources Center. For mouse interferon, 1 PR50 (VSV) unit was equivalent to about 2 units of the research reference mouse international standard (G002-904-511) from the National Institutes of Health (U.S.A.). As an internal control to monitor the sensitivity of the assay cells, we routinely included our own standard of chick and mouse interferon in all assays (Sekellick & Marcus, 1979a). Medium from mock-induced cells at no time contained any interferon.

RESULTS

Interferon induction by VSV: dose (multiplicity)-response (interferon yield) curves

Mutant tsG11(I)

Fig. 1 illustrates the results of a representative experiment in which a standard preparation of ts mutant G11(I) was used to induce interferon in aged chick embryo cells at a non-permissive temperature (40-5 °C). Characteristically, the amount of interferon in the medium at 24 h p.i. was critically dependent upon the m.o.i. Initially, an increase in multiplicity (measured as p.f.p.) led to a rapid increase in the yield of interferon, with the peak yield at multiplicity (m) of m0.5 ≈ 0.1. A further increase in multiplicity led to a steady decline in the yield of interferon. The second minor peak was not observed in three other experiments.

We note that revertants in the stock of tsG11 could not account for the i.f.p. activity recorded in Fig. 1 because the p.f.p. titres at 30 and 40-5 °C were 1.0 × 1010 and < 103/ml, respectively. Indeed, the titre of i.f.p., that is approx. 10 times the number of p.f.p., is of the order expected from the ratio of physical:infectious particles for VSV (Galasso, 1967) or for particles showing transcriptase activity (Bishop & Roy, 1971) or cell killing activity (Marcus & Sekellick, 1974).
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Fig. 3. Dose-response curve relating multiplicity of infection (p.f.p.) of VSV non-ts revertant R1 (T1026) to the interferon yield on mouse L(Y) cells at 37.5 °C.

Mutant non-ts R1 (T1026)

Fig. 2 shows that a non-ts revertant, R1, of tsT1026(I) tested under the same conditions generated a similar dose-response curve. In this case the peak yield of interferon was obtained at m_{n.f.p.} \approx 0.17. If we assume that the peak yield of interferon is achieved at m_{i.f.p.} \approx 1 (see Discussion; Marcus & Sekellick, 1977; Marcus et al. 1978; Marcus & Fuller, 1979) then the ratio i.f.p.:p.f.p. \approx 6.

Fig. 3 illustrates the dose-response curve obtained when VSV revertant R1 was used to induce interferon on a different cell type, the Youngner line of mouse L-cells, L(Y). In contrast to the results obtained from ‘aged’ chick embryo cells (Fig 1 and 2), the kinetics of interferon induction on mouse L-cells are similar to those usually observed in vitro (Fleischmann & Simon, 1974). These data best fit a model in which maximal yields of interferon are induced in cells infected with one or more i.f.p. Considering the nature of the dose-response curve and assuming a Poissonian distribution of i.f.p. in the cell population, we can calculate that at 63% of the maximum yield of interferon the cells were infected with an average of 1 i.f.p. Since this corresponds to m_{n.f.p.} = 0.14, the ratio i.f.p.:p.f.p. \approx 7. This ratio is very close to that obtained from the kinetically different dose-response curve generated on ‘aged’ chick embryo cells and leads us to conclude that both cell systems are detecting the same particles, and that there are about six to seven times more i.f.p. than p.f.p. in our stock of VSV revertant R1.

U.v. light survival curves for p.f.p. and i.f.p. activity of VSV

Fig. 4 illustrates the mean survival curve from three different experiments for the i.f.p. activity of VSV revertant R1. In all, individual points varied ± 50% from the mean value of the curve illustrated. The linear nature of the curve indicates that a single u.v. ‘hit’ to the virus genome inactivated i.f.p. activity; the D_{i.f.p.} (1/e, 37% survival) = 486 ergs/mm². The slope of the R1 i.f.p. survival curve relative to that for p.f.p. [included as an actinometer, where D_{p.f.p.} = 52.3 ergs/mm² (Marcus & Sekellick, 1975)], indicates that only about 11% of the genome need remain intact for the formation of an interferon inducer moiety. Fig. 4 also includes for comparison the u.v. survival curve for the i.f.p. activity of VSV DI-011 – a defective particle already possessing a potential interferon-inducer molecule of dsRNA (Marcus & Sekellick, 1977). We note that the u.v. dose which inactivates 90% of the i.f.p. capacity of VSV-R1 has very little effect on DI-011 i.f.p.
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Fig. 4. U.v. survival curves for VSV revertant R1: p.f.p. activity (○—●) and i.f.p. activity (□—○), and for VSV DI-011: i.f.p. activity (■—■) in aged CEC (1st passage). After different doses of radiation the i.f.p. activity was measured by treating cell monolayers with the same constant dilution of virus which, with unirradiated virus, induced maximal levels of interferon (m.f.p. = 1).

Fig. 5. Survival curves for VSV tsG11(I) p.f.p. activity (◇—◇) and i.f.p. activity (●—●), and for VSV DI-011 i.f.p. activity (■—■) in aged CEC after exposure of the virions to heat (50 °C).

Heat (50 °C) survival curves for p.f.p. and i.f.p. activity of VSV

Samples of VSV ts mutants G11(I) were held at 50 °C for various time intervals and the surviving fraction of p.f.p. and i.f.p. activities determined. The results of a representative experiment are presented in Fig. 5 and demonstrate that both activities are lost at the same linear rate, with an inactivation constant, \( K = 0.53 \text{ min}^{-1} \), a value in close agreement with that reported by Szilagyi & Pringle (1972), for the loss by heat (50 °C) of p.f.p. activity of tsG11. In this same experiment the i.f.p. activity of DI-011 was about seven times more resistant to heat inactivation. In two other experiments DI-011 i.f.p. showed almost complete resistance to heat.

DISCUSSION

In an earlier study we demonstrated that certain ts mutants and revertants of VSV were excellent inducers of interferon (Sekellick & Marcus, 1979a). From those studies we inferred that the capacity to produce an interferon-inducer moiety is intrinsic to VSV and may be expressed if macromolecular synthesis in the host cell is not compromised by the cell killing activity of the virus (Marcus & Sekellick, 1974, 1975; Marvaldi et al. 1977) and its capacity to inhibit cellular protein synthesis (Wertz & Youngner, 1970; McAllister & Wagner, 1976; Marvaldi et al. 1977, 1978). Indeed, all ts mutants which fail to inhibit cellular protein synthesis (psi⁻), or kill cells (ckp⁻) at non- or semi-permissive temperatures function as interferon-inducing particles (ifp⁺ phenotype) (Sekellick & Marcus, 1979a).

In the present study we have demonstrated that a single virion of VSV can induce a quantum yield of interferon—an attribute shared by other i.f.p. (Marcus & Sekellick, 1977; Marcus & Fuller, 1979; Fuller & Marcus, 1979). In the case of VSV, standard stocks may contain several times more i.f.p. than p.f.p., a characteristic which may have
important implications in the initiation and/or maintenance of persistent infection and cell persistence through the interferon system (Sekellick & Marcus, 1978, 1979a, 1980).

The observed u.v. dose for inactivating i.f.p. activity \( (D^0_{i.f.p.} = 486 \, \text{ergs/mm}^2) \) appears to rule out formation of an interferon-inducer moiety from the 48-base leader sequence and its complement; a structure comparable in size and base sequence to leader RNA (Colonno & Banerjee, 1976, 1977, 1978) would be expected to generate a \( D^0_{i.f.p.} \) value at least 10-fold greater than that observed.

The mandatory single initiation site for transcription by VSV and its gene order (Ball & White, 1976; Abraham & Banerjee, 1976), coupled with the observed \( D^0_{i.f.p.} \) value dictates that to achieve i.f.p. activity transcript synthesis must start at the leader sequence (Colonno & Banerjee, 1977) and proceed, at the minimum, about two-thirds of the way into the \( N \) gene. Thus, i.f.p. activity can be expressed by a virion incapable of replicating, or of synthesizing any virus proteins. A virion of VSV u.v.-irradiated to this extent may be described phenotypically as: \( \text{tra}^+, \text{ifp}^+, \psi^-\text{ckp}^-, \text{rep}^-, \text{pfp}^- \).

Most investigators have concluded that dsRNA is the interferon-inducer moiety of viruses and that some virus synthetic events are required to synthesize a threshold amount of this dsRNA (for example, Burke, 1973; Johnston & Burke, 1973; Atkins et al. 1974; Atkins & Lancashire, 1976). We have recently provided compelling evidence that dsRNA is indeed the inducer and that a single molecule per cell represents that threshold (Marcus & Sekellick, 1977; Marcus & Fuller, 1979; Fuller & Marcus, 1979).

In the light of these studies and the present data we suggest that the following events may lead to the formation of a molecule of dsRNA, the presumed interferon inducer, by VSV i.f.p. We hypothesize that with each successive polymerase molecule advancing during primary transcription (Bishop & Smith, 1977), and as a reflection of the normal half-life of \( N \) in the cell, there is a certain probability that a molecule of \( N \) protein (and/or NS and L) will dissociate from the ribonucleoprotein complex, leaving a base free for complementary pairing. Thus, after a few rounds of polymerase movement in which the dissociated \( N \) protein is not replaced, an RNA helix may form with sufficient stability to be sensed by the cell as an interferon inducer.

Two lines of evidence support this hypothesis. First, the RNA in defective-interfering particles (d.i.p.) which contain a single molecule of \([\pm]\)RNA that is covalently linked and self-complementary, assumes within the cell a configuration which functions as an interferon-inducer (Marcus & Sekellick, 1977). Presumably, this is a helical structure since a \([-]\)RNA d.i.p. lacking significant self annealing, (beyond that attributable to the 3' and 5' termini) but with the same structural proteins, fails to function as an i.f.p. (Marcus & Sekellick, 1977). Since the i.f.p. activity of the \([\pm]\)RNA d.i.p. is acquired in the absence of helper virus, any molecules of \( N \) (or NS or L) protein lost by dissociation could not be replaced. By our model, this condition would ultimately lead to base-pairing and the formation of a stable helix. Secondly, most of the \( ts \) mutants with an \( \text{ifp}^+ \) phenotype are defective in \( N \), NS or L proteins of the RNP complex. For example, the \( N \) protein of \( tsG41(IV) \) synthesized at non-permissive temperatures is demonstrably less stable than wild-type \( N \) (Knipe et al. 1977) and, by our model, could not replace functional \( N \) lost by dissociation from the input RNP. Although the actual situation may be more complicated than the simple model proposed, it provides a basis for further experimentation.

Our studies have identified an excess of VSV i.f.p. over p.f.p. and suggest that a virus stock preparation may contain potential inducers of interferon that may be masked by the lethal action of the virus. However, their presence might have profound effects on the outcome of a real virus infection as suggested by the \textit{in vivo} studies of Gresser et al. (1976). With so many RNA molecules of complementary sequence that can be isolated from virus-infected cells and a threshold for i.f.p. activity of one dsRNA molecule per cell, it is not surprising that most viruses can induce interferon.
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REFERENCES


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