Interferon Induction by Viruses. XVII. Non-temperature-sensitive Mutations Regulate Interferon Induction by Vesicular Stomatitis Virus

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SUMMARY

Wild-type (wt) strains of vesicular stomatitis virus (VSV) strain Indiana are poor to non-inducers of interferon (IFN) which express IFN induction-suppressing activity. At non-permissive temperatures, temperature-sensitive (ts) mutants of this virus are either like their wt parents, or they are good to excellent inducers of IFN. IFN inducibility and IFN induction-suppressing activity are mutually exclusive phenotypes in VSV-Indiana. With one exception, all Orsay ts mutants derived by A. Flamand (CNRS, Gif-sur-Yvette, France), representing the five complementation groups, were poor to non-inducers of IFN and were also capable of suppressing IFN induction by other viruses. In contrast, all Glasgow ts mutants derived by C. R. Pringle (University of Warwick, Coventry, U.K.) were excellent inducers of IFN. We demonstrate that this difference in acquisition of IFN inducibility relates primarily to the origin of the mutations; spontaneous for Orsay, and mutagen-derived for Glasgow. Tests with newly generated spontaneous and mutagen-derived mutants, and temperature-stable revertants of IFN-inducing ts mutants indicate that IFN inducibility results from non-ts, multiple mutations rarely acquired spontaneously, but generated frequently upon mutagenesis with 5-fluorouracil. The capacity of VSV-Indiana to induce IFN is considered intrinsic to the virus, but is only manifested when the dominant IFN induction-suppressing phenotype is lost through mutagenesis. Thus, non-ts mutations appear to regulate the expression of the IFN induction-suppressing phenotype, and hence the IFN inducibility of VSV-Indiana.

INTRODUCTION

Wild-type (wt) vesicular stomatitis virus (VSV) of the Indiana serotype generally is considered a poor or non-inducer of interferon (IFN) (Wagner et al., 1963; Wagner & Huang, 1966; Wertz & Youngner, 1970; Sekellick & Marcus, 1979; Francoeur et al., 1980). However, temperature-sensitive (ts) mutants of VSV-Indiana are either like their wt parent in being poor to non-inducers of IFN, or they are good to excellent inducers at non-permissive temperatures (Sekellick & Marcus, 1979). Stocks of VSV that are non-inducers of IFN also display another attribute: they suppress IFN induction in cells co-infected with a virus which is otherwise competent to induce IFN. Functionally, these stocks consist of IFN induction-suppressing particles (ISPs) (Marcus & Sekellick, 1985). This IFN induction-suppressing activity (termed the isp+ phenotype) is invariably dominant to IFN-inducing activity (the ifp+ phenotype). These two phenotypes are mutually exclusive in strains of the Indiana serotype. Thus, an IFN-inducing particle (IFP) (phenotype ifp+,isp-) does not suppress IFN induction, and an ISP (ifp-,isp+) does not induce much, if any, IFN (Marcus & Sekellick, 1985, 1987).

Why are some ts mutants of VSV excellent inducers of IFN at non-permissive temperatures and others not? If the IFN-inducing capacity of a VSV ts mutant was linked to the nucleotide change(s) responsible for a particular ts defect, then the IFP activity might be expected to correlate with the gene representing a specific complementation group. An examination of ts mutants representing the five complementation groups of VSV-Indiana revealed no such
correlation (Sekellick & Marcus, 1979). However, a striking relationship was observed between the IFN-inducing capacity of ts mutants and their parental origin. With one exception, all of the ts mutants tested that derived from the Orsay wt (Flamand, 1970) were poor to non-inducers of IFN. In contrast, all of the ts mutants tested of Glasgow parentage (Pringle, 1970) were good to excellent inducers of IFN (Sekellick & Marcus, 1979).

There did not appear to be anything intrinsically unusual about the nature of the ts defects between the two groups of ts mutants, since Flamand & Pringle (1971) demonstrated complete homology in complementation tests between the independently derived collections of their respective mutants. However, examination of the procedures used by Flamand (1970) and by Pringle (1970) to obtain the Orsay- and Glasgow-derived ts mutants, respectively, revealed that the former were isolated from plaques as spontaneous ts mutants, whereas the latter were obtained after chemical mutagenesis. One apparent exception was tsOS5(I). Although this mutant was derived from an N-nitrosoguanidine-grown stock, it actually may have been of spontaneous origin, since the proportion of ts mutants (5-0%) obtained after treatment was not significantly different from that found in a control untreated virus stock (4-3%) (A. Flamand, personal communication).

On the basis of the different modes of origin of the Orsay and Glasgow mutants, we hypothesized that non-ts mutations introduced into the Glasgow genome of VSV with mutagens may have created the ifp+ phenotype. This most likely occurs by eliminating the normally dominant isp+ phenotype intrinsic to parental wt viruses (Marcus & Sekellick, 1985, 1987). Compared to ts mutants obtained by mutagenesis, spontaneously derived ts mutants might contain fewer of the nucleotide changes postulated as necessary to eliminate the isp+ phenotype. Once this phenotype was eliminated, the ifp+ phenotype could then be expressed. Were this simple hypothesis correct, spontaneous ts mutants, regardless of their origin, should almost always display the same phenotype as the parental wt (ifp+,isp+), whereas those derived by mutagenesis might contain a class of good IFN inducers (ifp+,isp-). Furthermore, if the ifp+ phenotype is dependent upon mutations that are independent of those responsible for the ts phenotype, then wt revertants selected from any IFN-inducing ts mutant might not be expected to revert concomitantly to a loss of IFN inducibility and a return to the isp+ phenotype. Revertants of this sort would be expected to retain IFN-inducing capacity, especially if multiple mutations, or an infrequent one, were required to create the ifp+,isp- phenotype. This communication describes experiments to test predictions based on this hypothesis.

METHODS

Cells and media. A description of the cell cultures and the materials and methods used for their preparation and maintenance have been described in detail (Sekellick & Marcus, 1979, 1985; Marcus et al., 1983). Primary chick embryo cells were prepared from 10-day-old Utility grade embryonated eggs (Spafas, Norwich, Conn., U.S.A.), seeded in NCI medium containing 6% calf serum (also referred to as attachment solution), and aged in vitro to enhance the cells' capacity to respond to inducers of IFN (Sekellick & Marcus, 1986). The clone of GMK–Vero cells used to grow and plaque assay viruses did not produce IFN in response to virus infection. BHK cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum and 10% tryptose phosphate broth.

Viruses: source, preparation and assay. The origin, growth and plaque assay of VSV-Indiana wts of Glasgow and Orsay origin, and their ts mutants have been described (Sekellick & Marcus, 1979). We now note that tsTI026 was obtained by T. Nakai and A. F. Holloway following mutagenesis of the Toronto-HR wt (C. P. Stanners, personal communication). Other wt VSVs were obtained as follows: Mass. and San Juan provided by H. Lodish, and MS from R. A. Lazzarini.

IFN induction and assay. Detailed protocols have been described for the induction and assay of IFN in monolayers of aged primary chick embryo cells (Sekellick & Marcus, 1986), including a description of the generation and analysis of IFN induction dose (multiplicity)-response (IFN yield) curves used to detect and measure IFN-inducing particles (Marcus, 1986). IFN assays utilized a computer-driven Bio-Tek Instruments' 96-well microtitre plate reader to generate IFN titres (Sekellick & Marcus, 1986). The computer program was developed by Mary K. Donnelly.

Ts mutants: isolation and screening. The isolation and screening of ts mutants followed in general procedures previously described (Flamand, 1970; Pringle, 1970; Youngner et al., 1976). However, all stocks of virus were grown in a clonal line of GMK–Vero cells that does not respond to inducers of IFN (Marcus & Sekellick, 1976),
Non-ts mutations control IFN induction by VSV

Data on the IFN-inducing capacity of wt and ts mutants of VSV have been collected over several years. A representative experiment is presented in Table 1. IFN yields depict maximum values obtained from full dose–response curves as discussed in Methods. In the experiment illustrated, inductions for a set of ts mutants or the wt strains were carried out in the same lot of aged chick cells so that relative maximum yields of IFN could be compared directly. This procedure was used to circumvent the lot-to-lot variation in absolute yields of IFN observed as chick embryo cells age in vitro.

Consistently, all of the Glasgow-derived mutants were excellent inducers of IFN, as was one of the Orsay mutants, tsO45(V). In contrast, all six wts and the remaining six ts mutants of Orsay origin were poor to non-inducers of IFN, producing less (often much less) than 5% of the IFN induced by the Glasgow ts mutants. Extremes in IFN-inducing capacity were observed even within the same complementation group. Thus, two of three group V Orsay mutants were very poor inducers, accumulating less than 0.2% of the IFN yield of tsO45(V).

The IFN-inducing capacity of spontaneously derived ts mutants of Glasgow and Orsay origin

Four-hundred and eighty plaques of Glasgow wt virus, and 500 plaques of Orsay wt virus, all grown and plated in the absence of mutagen, were picked and screened for the ts phenotype as described. Twenty-six provisional Glasgow mutants appeared in the first screening. Upon regrowth of the virus and a second screening, six (6/480 = 1.2%) gave PFP ratios at 40 °C : 30 °C of < 10⁻³. These were designated tsSG (Storrs-derived from Glasgow parent). When 500 plaques were picked from the Orsay parent in the absence of a mutagen, 18 provisional ts
Table 1. **IFN-inducing capacity of VSV wild-types and ts mutants**

<table>
<thead>
<tr>
<th>Parental type (Indiana)</th>
<th>Wt or ts mutant</th>
<th>IFN yield (U/10^7 cells)*</th>
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</thead>
<tbody>
<tr>
<td>Orsay Wt</td>
<td>&lt; 10</td>
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</tr>
<tr>
<td>San Juan Wt</td>
<td>&lt; 10</td>
<td></td>
</tr>
<tr>
<td>Mass. Wt</td>
<td>280</td>
<td></td>
</tr>
<tr>
<td>Toronto (HR) Wt</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Glasgow Wt</td>
<td>420</td>
<td></td>
</tr>
<tr>
<td>MS Wt</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Glasgow G11(I)</td>
<td>8700</td>
<td></td>
</tr>
<tr>
<td>G114(I)</td>
<td>26500</td>
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</tr>
<tr>
<td>G22(II)</td>
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</tr>
<tr>
<td>G31(III)</td>
<td>26500</td>
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<tr>
<td>G41(IV)</td>
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<tr>
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<tr>
<td>O23(III)</td>
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<tr>
<td>O100(IV)</td>
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<tr>
<td>O44(V)</td>
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<tr>
<td>O110(V)</td>
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</tr>
<tr>
<td>O45(V)</td>
<td>26500</td>
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</tr>
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</table>

* IFN yields represent the maximum output (quantum yield) of IFN obtained by infecting aged primary chick embryo cells for 24 h at 40-5 °C, and were determined from a full dose-response curve (Marcus, 1986). Data from the Glasgow and Orsay mutants are summarized in part from Sekellick & Marcus (1979).

mutants were isolated. After regrowth of the virus and a second screening, 12 (12/500 = 2.4%) gave PFP ratios at 40 °C:30 °C of < 10^-3. These were designated tsSO (Storrs-derived from Orsay parent).

Table 2 presents the IFN-inducing capacity of these spontaneously derived ts mutants. Ts mutant G41(IV) was included in each experiment as a standard for IFN induction in order to normalize the IFN-inducing capacity of viruses on different lots of chick embryo cells. Again the Orsay wt induced much less IFN than the Glasgow wt. Furthermore, all six of the spontaneously derived tsSG (Storrs/Glasgow) mutants were poor inducers of IFN, with five of the six mutants inducing significantly less than the Glasgow wt. The best inducer produced about the same amount of IFN as its wt parent, representing < 4% of the control yield of IFN from a standard inducer, tsG41. All 12 of the spontaneously derived tsSO (Storrs/Orsay) mutants also were poor inducers of IFN, even though two mutants induced significantly more IFN than the wt parent. Normalizing the yield of IFN between the two different lots of chick embryo cells, and recognizing the twofold error characteristic of IFN assays, revealed that the mean yield from the tsSG mutants [274 units (U)/10^7 cells] was essentially equivalent to that from the tsSO mutants (218 U/10^7 cells).

The species of host cell and the origin of wt VSV influence the effects of 5-FU

Initial experiments with 5-FU as a mutagen failed to produce a significant increase in the frequency of ts mutants when Orsay wt virus was exposed to the mutagen in Vero cells (data not shown), even at concentrations of the drug that exceeded those used routinely by other investigators (see Pringle, 1970). Further study revealed that the efficacy of the 5-FU was affected by both the host cell and the parental origin of the wt virus. These effects are illustrated in Fig. 1, where the fraction of control yields of VSV PFP are plotted as a function of 5-FU concentration during virus replication on Vero, primary chick embryo and BHK cells. A comparison of data in Fig. 1 (a) (Orsay wt) with those in Fig. 1 (b) (Glasgow wt) reveals that the replication of Glasgow wt was affected significantly more by the action of 5-FU than was the replication of Orsay wt. For example, with BHK cells as hosts, Orsay wt required about 20 times more 5-FU than Glasgow wt to achieve a 10-fold reduction in the yield of infectious virus. Furthermore, with Vero cells as hosts, 5-FU had little, if any, effect on the replication of either wt, thus providing an explanation for our failure to obtain a significant increase in the frequency
Table 2. IFN-inducing capacity of spontaneously derived VSV ts mutants

<table>
<thead>
<tr>
<th>Parental type (Indiana)</th>
<th>Wt or ts mutant</th>
<th>IFN yield (U/10^7 cells)*</th>
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<tr>
<td>Glasgow (standard)†</td>
<td>G41(IV)</td>
<td>34600</td>
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<td>Wt</td>
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<td>SG240A</td>
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<td></td>
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<td></td>
<td>SG415A</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>SG216A</td>
<td>1130</td>
</tr>
<tr>
<td>Glasgow (standard)†</td>
<td>G41(IV)</td>
<td>19460</td>
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<td></td>
<td>SO299</td>
<td>1020</td>
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</table>

* See footnote to Table 1.
† The Glasgow and Orsay mutants were each tested in a different lot of aged chick embryo cells. Inclusion of the same stock of tsG41 as a standard IFP permits normalization of IFN yields between lots of primary chick embryo cells. Thus, the cells used to test the tsSG mutants produced about twice as much IFN upon induction with tsG41 as those used to test the tsSO mutants. Variation is attributed to differences in the rate of 'ageing' of primary cells in vitro as they develop an enhanced capacity to produce IFN (Sekellick & Marcus, 1985).

of Orsay ts mutants with this cell line as a host. The basis of these differences has not been pursued. However, it is clear that the replication of VSV was affected most by the action of 5-FU with BHK cells as hosts, and that replication of the Glasgow strain of VSV was more sensitive to the action of 5-FU than that of the Orsay strain. We note that Pringle (1970) used BHK cells for the generation of mutagen-derived ts mutants of Glasgow origin and also reported differences in the sensitivity of various serotypes of VSV to 5-FU (Pringle, 1975). Chick embryo cells were used to isolate the spontaneous ts mutants of Orsay origin (Flamand, 1970).

The IFN-inducing activity of mutagen-derived ts mutants

A stock of Orsay wt virus was grown at 30 °C in BHK cells in the presence of 500 μg/ml 5-FU as described in Methods. This treatment resulted in a 70% reduction in the yield of virus. From the surviving virus yield, 240 plaque isolates were picked and screened for the ts phenotype. Twenty-six plaques passed the initial screen and were grown to full titre at 30 °C. Ten of these initial isolates (10/240 = 4.2%) contained virus that gave PFP ratios at 40 °C:30 °C of ≤10^{-3}. These mutants were used to generate IFN induction dose-response curves from which maximal yields of IFN were calculated. These data are shown in the right-hand part of Table 3, and reveal that all of the 10 mutants tested induced less than 5% of the IFN yield of the standard inducer, tsG41.

Wt Glasgow virus was grown under the same conditions, but in the presence of only 100 μg/ml 5-FU. This resulted in an 85% reduction in infectious titre, reflecting the greater sensitivity of the Glasgow wt to 5-FU (Fig. 1). Thirty-six plaque isolates from this mutagenized stock passed the initial screen. Of these initial isolates, 19 (19/240 = 7.9%) produced PFP ratios at 40 °C:30 °C of ≤10^{-3}, and were used to generate full IFN induction curves from which the maximum yields of IFN were calculated (shown in the left-hand part of Table 3). Ten of these 19 tsSG mutants induced 100-fold less, and six induced 20-fold less, IFN than the standard inducer,
Fig. 1. The replication of wt VSV in different cells as a function of 5-FU concentration. Cell monolayers were infected with wt VSV Orsay (a) or Glasgow (b) at an $m_{mp}$ of 0.5, and incubated at 30 °C in medium containing 5-FU for 24 h. Cell types used were Vero (●), primary chick embryo, 1 day in culture (□) and BHK (●).

Fig. 2. IFN induction dose (multiplicity)-response (IFN yield) curves for VSV wt, prototype IFN-inducing $ts$ mutants, and representative temperature-stable revertants. IFN induction was carried out in monolayers of primary chick embryo cells aged for 7 days in vitro. Following infection (induction), incubation was for 24 h at 40.5 °C. IFN was assayed as acid-stable material as described in Methods. The titre in PFP/ml at 30 °C and 40 °C, respectively, is as follows: (a) Orsay-derived wild-type $3.2 \times 10^9, 1.3 \times 10^9$ (●), $tsO45 3.6 \times 10^9, 2.1 \times 10^9$ (●), $tsO45R10 5.1 \times 10^9, 4.9 \times 10^9$ (□), $tsO45R9 6.3 \times 10^9, 5.9 \times 10^9$ (■), and (b) Glasgow-derived wt $7.2 \times 10^9, 3.6 \times 10^8$ (●), $tsG41 4.4 \times 10^9, 5.7 \times 10^8$ (●), $tsG41R9 2.3 \times 10^9, 4.8 \times 10^8$ (□), $tsG41R13 1.6 \times 10^9, 7.9 \times 10^8$ (■).
Table 3. *IFN*-inducing capacity of mutagen (5-FU)-derived *ts* mutants of *VSV*-Indiana

<table>
<thead>
<tr>
<th>Wt or mutant</th>
<th>IFN yield (U/10^7 cells)</th>
<th>Wt or mutant</th>
<th>IFN yield (U/10^7 cells)</th>
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<tr>
<td>Wt</td>
<td>202</td>
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<tr>
<td>G41*</td>
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<td>G41*</td>
<td>11620</td>
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<td>SG2</td>
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* Used as standard inducer; see footnote to Table 1.

tsG41. The three remaining mutants were good inducers of IFN. One mutant (tsSG2) induced as much IFN as the standard inducer, thus resembling the *ifp*⁺ phenotype of the *ts* mutants isolated by Pringle (1970).

The IFN-inducing capacity of mutants *tsO45*, *tsG41*, and their temperature-stable (wt-like) revertants

Insight into the genetic alteration(s) responsible for acquisition of IFN inducibility may be gained by obtaining revertants that have lost that capacity. To this end, spontaneous temperature-stable revertants of two excellent inducers of IFN from complementation groups showing significant reversion rates were examined for IFN-inducing capacity. Wild-type revertants from *tsO45* and *tsG41* were obtained at frequencies of 8 × 10⁻⁵ and 1 × 10⁻⁴, respectively. These values are in accordance with those reported by Pringle (1975). Our failure to obtain revertants from mutants in complementation groups I and II also is in keeping with the experience of others (Pringle, 1975). We did not isolate revertants from group III mutants, but they have been reported (Morita *et al.*, 1987). Examination of temperature-stable revertants, 14 from *tsG41* and nine from *tsO45*, revealed that all but one retained IFN-inducing capacity, and several exceeded that of their *ts* parent (Table 4). Preliminary data suggest that the only poor inducer of IFN, SGR1, may display an *isp*⁺ phenotype close to that of the wt. Full IFN induction dose-response curves for two of the *tsO45* revertants with high 40 °C:30 °C ratios were compared with *tsO45* and its parent wt (Fig. 2a). Analysis of these curves reveals that PFP and IFPs are present in essentially equal numbers (Marcus, 1986). Revertants R9 and R10 of *tsO45* consistently induced more IFN than their parent, in spite of expressing a temperature-stable phenotype.

Similar results were obtained with wt revertants from *tsG41*. All but one of the temperature-stable revertants retained their capacity to induce IFN. Two revertants were characterized in detail with full IFN induction dose-response curves as shown in Fig. 2(b). Analysis of these curves again reveals a ratio of PFP:IFP of approx. 1.
Table 4. IFN-inducing capacity of temperature-stable revertants of ifp+ mutants tsG41 and tsO45

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<th>Glasgow-derived</th>
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<td>Wt or mutant</td>
<td>IFN yield (U/10^7 cells)</td>
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* Plaque isolates of VSV stocks were screened for their IFN-inducing capacity by infecting monolayers of chick embryo cells aged in vitro for 9 days with a single common dilution (1:50) as described in Methods. These results do not necessarily reflect the maximum yield of IFN that the virus can induce; this can be determined only by analysing the yields in a full dose-response curve. This rapid screen is used to identify stocks capable of inducing IFN. All low or poor inducers must be confirmed with a full dose-response curve that will distinguish between viruses that are intrinsically good inducers at one particle per cell, but which at higher multiplicities will score as non- to poor inducers because of the nature of the dose-response curve (see Marcus, 1986). Additional studies confirmed that SGR1 was a poor inducer of IFN capable of suppressing IFN in a cell co-infected with an IFP. Thus, SGR1 may represent a revertant closer to the wt isp+ phenotype.

DISCUSSION

All of the Glasgow-derived ts mutants of VSV-Indiana isolated by Pringle (1970), representing four of the five complementation groups, induce IFN with high efficiency at 40.5 °C in aged chick embryo cells (Sekellick & Marcus, 1979; Table 1). Thus, it appeared that all mutations leading to the ts defect concomitantly might lead to the acquisition of IFN inducibility. However, studies extended to include the ts mutants of Orsay origin (Flamand, 1970) revealed that these mutants were poor or non-inducers, with the exception of one of three mutants in complementation group V (Sekellick & Marcus, 1979; Table 1). Clearly, no direct relationship exists between the acquisition of the ts phenotype and the acquisition of IFN-inducing capacity. This prompted us to consider the origin of the mutations, spontaneous for Orsay and mutagen-derived for Glasgow, as a contributing factor. Indeed, data presented here demonstrate that all but one of the 18 (6%) ts mutants derived spontaneously from either Orsay or Glasgow wt parents were poor to non-inducers of IFN (Tables 1 and 2). Yet, from 34 mutagen-derived ts mutants examined in detail, eight were good to excellent inducers of IFN (24%) (Tables 1 and 3). While most mutagen-derived mutants are poor to non-inducers, the few that do induce IFN often do so with different quantum yields (Tables 3 and 4; Fig. 2a), suggesting that cumulative effects of mutations may regulate expression of the ifp+ phenotype.

These data demonstrate that acquisition of the ts phenotype, is not a sufficient condition for generating a virus capable of inducing IFN. Instead, it appears that nucleotide changes beyond those required to create a ts mutant are needed for expression of the ifp+ phenotype. This view is supported by the observation that reversion of ts mutants expressing the ifp+ phenotype to a temperature-stable, wt-like, phenotype is not accompanied by loss of IFN-inducing capacity in 22 of the 23 revertants tested. Thus, reversion to temperature stability is not often accompanied by reversion to the isp+ phenotype that is characteristic of all wt isolates of VSV-Indiana examined thus far. Indeed, revertants may contain mutations that enhance IFN-inducing capacity (Table 4).
These IFN-inducing, temperature-stable revertants are similar to T1026R1 (also derived from an IFN-inducing, ts parent, tsT1026) (Stanners et al., 1977), and possibly like more recent isolates (Francoeur et al., 1987). However, the IFN-inducing capacity of these recently isolated revertants was not measured directly; it was inferred from their mode of isolation.

Collectively, these data demonstrate conclusively that expression of a temperature-stable phenotype and an ifp+ phenotype are not mutually exclusive. This contrasts with the mutual exclusiveness of the ifp+ and isp+ phenotypes in true wt strains of VSV-Indiana examined to date (Table 1; and Marcus, 1982). Clearly, mutations leading to the ifp+ phenotype are not the same as those responsible for the ts phenotype. Most likely these temperature-stable revertants are not true wts, in that none induced cytopathic changes as rapidly as the wt parent (unpublished observations).

The capacity to suppress IFN induction is shared by all of the ts mutants that are poor to non-inducers of IFN as ascertained by co-infecting cells with an IFP and the test mutant at an mPP of 5 (data not shown).

The isp+ phenotype is dominant to the ifp+ phenotype (Marcus & Sekellick, 1985), and these two are mutually exclusive as expressed in all strains of VSV-Indiana examined thus far. (This is not true for the New Jersey serotype of VSV; Marcus et al., 1987.) Consequently, the proper designations for the phenotypes of VSV-Indiana particles that express IFN-inducing or IFN induction-suppressing activity are ifp+,isp- and ifp-,isp+, respectively.

Dominance of the isp+ phenotype prompts a different perspective on the origin of the ifp+ phenotype. Rather than considering that the ifp+ phenotype is a consequence of genetic changes that lead to the acquisition of an IFN-inducing capacity, it may be more accurate to think in terms of IFN induction resulting from genetic changes that produce a loss or repression of the isp+ phenotype, thereby allowing expression of an otherwise latent IFN-inducing phenotype. From this view, all wt strains of VSV possess the intrinsic property of inducing IFN by virtue of their formation of dsRNA during normal replication. Because of evidence that dsRNA-dependent enzymes can be activated during VSV infection (Rice et al., 1985), it appears that replication of VSV results in the formation of dsRNA. However, IFN induction by VSV in aged chick embryo cells at 40.5 °C does not require replication or amplified RNA synthesis for formation of the IFN inducer moiety. The only requirement is for transcription of about 10% of the 3' most proximal portion of the RNA genome (Marcus & Sekellick, 1980).

Suppression of IFN induction by VSV requires transcription of only about 0.5% of the genome (Marcus & Sekellick, 1978). Based on evidence and reasoning developed earlier (Marcus & Sekellick, 1985, 1987), we postulated that the IFN induction-suppressing activity of VSV most likely resides in the action of the product of the 47-base leader RNA sequence at the extreme 3' end of the genome, i.e. plus-strand leader (Banerjee, 1987). Thus, mutations affecting the expression or activity of this plus-strand leader RNA may play a role in the loss of the isp+ phenotype (and the accompanying expression of the ifp+ phenotype), especially in view of its inhibitory effect on cellular polymerase II (McGowen et al., 1982; Grinnell & Wagner, 1984).

Collectively, these results strengthen our conclusion that the probability of generating a ts mutation is significantly higher than that of generating a mutation(s) that leads to loss of the isp+ phenotype and the subsequent expression of IFN inducibility. In general, the probability of obtaining mutants may be higher for wt Glasgow than Orsay because the former may contain a less discriminating polymerase (Fig. 1). The scarcity of revertants to the isp+ phenotype suggests that multiple mutations are involved, or an infrequent one, in the loss of IFN induction-suppressing activity. However, with some evidence to the contrary (Rae & Elliott, 1986; Gallione & Rose, 1985), this assumption should be tested. Future studies will attempt to define the genetic changes and protein functions that regulate IFN induction, and its suppression.

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Non-ts mutations control IFN induction by VSV


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