Interferon Induction by Viruses. X. A Model for Interferon Induction by Newcastle Disease Virus

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(Accepted 5 August 1983)

SUMMARY

Unirradiated Newcastle disease virus (NDV, strain AV) induced high levels of interferon (IFN) in primary chick embryo cells if the cells were ‘aged’ in vitro for 6 to 7 days. Dose (multiplicity)-response (IFN yield) curves, carried out in the presence of anti-NDV serum to prevent cycling infection, revealed that stocks of NDV-AV contain about sevenfold more IFN-inducing particles (IFP) than infectious particles (PFP). These non-infectious IFP were responsible for nearly all IFN induction in ‘aged’ cells, since PFP were determined to be incapable of inducing IFN. In contrast, with mouse L(Y) cells as hosts, about one-third the number of particles as there are PFP appeared to score as IFP. Heat and u.v. radiation (254 nm) inactivated NDV IFP and PFP activity at the same rate whether tested in chick or mouse cells, implying that virion-associated transcription is required to induce IFN. A model is proposed to account for the generation of IFN-inducing particles from infectious NDV following u.v. irradiation, and their subsequent inactivation at high doses of radiation. The model defines a series of u.v. targets in the NDV genome that regulate the expression of IFN-inducing particle activity in ‘unaged’ chick embryo cells.

INTRODUCTION

Newcastle disease virus (NDV) is generally considered to be an excellent inducer of interferon (IFN), and has been the subject of extensive studies designed to define the nature of its inducer and its mechanism of IFN induction (for review, see Johnston & Burke, 1973; and recent references in Vilcek & Kohase, 1977; Kowal & Youngner, 1978; Kohase & Vilcek, 1979; Brown et al., 1980; Burke, 1981/82; Marcus, 1983). Interferon induction by NDV is characterized by two distinct host cell-related features: (i) with permissive chick embryo cells as hosts, stocks of NDV function poorly as inducers of IFN, but acquire excellent inducing capacity when inactivated by various physical, chemical or biological methods, and (ii) with non- or semi-permissive mouse L, or other mammalian cells, stocks of otherwise infectious NDV function as excellent inducers of IFN (Burke & Isaacs, 1958; Cantell et al., 1962; Wagner, 1964; Ho & Breinig, 1965; Wheelock, 1966; Youngner et al., 1966; Kohno & Kohase, 1969; Kohno et al., 1969; Stewart et al., 1971; Sheaff et al., 1972; Meager & Burke, 1972; Lomnicz, 1973; Fleischmann & Simon, 1974; Azuma, 1976; Brown et al., 1980).

In this communication we demonstrate that (i) NDV stocks can be defined quantitatively in terms of their IFN-inducing particle (IFP) activity by analysing dose (multiplicity)-response (IFN yield) curves (Marcus, 1982), (ii) stocks of NDV contain a heretofore unrecognized activity, non-infectious IFP, that are excellent inducers of IFN in primary chick embryo cells.

Abbreviations. PFP, Plaque-forming particle; IFP, interferon-inducing particle; ISP, interferon induction-suppressing particle; ifp⁺, ifp⁻, the phenotype designation for virus particles capable, and incapable, respectively, of inducing interferon. (Phenotypic expression can depend upon, for example, the host cell, induction temperature, and the presence of co-infecting virus.) CEC, Chick embryo cells; L(Y), a line of mouse L cells obtained from J. S. Youngner; NDV, Newcastle disease virus.

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(CEC) if the cells are 'aged' in vitro for several days, (iii) this IFP activity is present in about sevenfold excess over that of plaque-forming particles (PFP), (iv) infectious NDV does not induce IFN in chick embryo cells, even in 'aged' cells, and (v) only about one-third of the number of particles as there are PFP appear to register as IFP on mouse L cells. In addition, we postulate that stocks of NDV contain some particles which function antagonistically to IFP; these are termed IFN induction-suppressing particles (ISP) (Marcus, 1982).

We confirm earlier observations that formation of an IFN inducer by NDV requires primary transcription, and propose a model which quantitatively accounts for the acquisition of IFN-inducing capacity by NDV upon exposure to u.v. radiation, when tested in 'unaged' chick embryo cells.

METHODS

Cells and medium. The preparation and aging of monolayers of primary chick embryo cells has been described previously (Carver & Marcus, 1967; Marcus & Sekellick, 1977; Winship & Marcus, 1980). Briefly, monolayers of primary chick embryo cells are allowed to age 6 days (without medium change) at 37.5 °C in attachment solution (NCI medium plus 6% calf serum). While young chick embryo cells were poor IFN producers when stimulated by viruses, monolayers aged 6 days in vitro produced up to 100-fold more IFN (Carver & Marcus, 1967; P. I. Marcus et al., unpublished results). The preparation of mouse cells [L(Y); a line of L cells obtained from J. S. Youngner] has been described by Sekellick & Marcus (1978). These cells have been characterized as superior producers of interferon (Winship & Marcus, 1980; Marcus et al., 1981; Sekellick & Marcus, 1982). Cells aged 4-5 days in vitro and maintained at 37.5 °C produced maximum levels of interferon (Marcus et al., 1981; Winship & Marcus, 1981; Guidon, 1982).

Viruses: source and stock preparation. The AV (Australia–Victoria, 1932) strain of NDV was provided originally by Dr L. E. Hightower. Stocks were prepared as described (Sekellick & Marcus, 1978). Cholioallantoic fluid was clarified by centrifugation at 2000 rev/min for 10 min. The non-purified virus stocks were maintained frozen at −70 °C and either used directly or diluted in culture medium prior to addition to cell monolayers. The source and preparation of vesicular stomatitis virus (VSV) Indiana wild-type (HR) has been described previously (Marcus & Sekellick, 1974). Mengovirus (is + ) was obtained originally from E. Simon and prepared as described by Marcus et al. (1981).

Antiserum: stock preparation. NDV antiserum used in this study was prepared in New Zealand White rabbits using the procedure of Hunt & Marcus (1974), with several modifications. Three 0.4 ml injections of u.v.-inactivated NDV (each equivalent to $10^9$ NDV PFP before inactivation), were given 1 week apart into the lateral ear veins. NDV-specific antiserum was obtained from blood collected 5 days after the last injection. The antiserum was heat-inactivated (56 °C, 1 h) and adsorbed three times successively on confluent monolayers of CEC to remove any antacellular activity. A 1:100 dilution of antiserum produced a 100-fold reduction in NDV PFP titre when incubated with u.v. at 30 min at 37 °C.

Inactivation of virus by u.v. radiation. Stocks of NDV were diluted in attachment solution (NCI medium plus 6% calf serum) (Marcus & Carver, 1965) and exposed to u.v. radiation (254 nm) as previously described (Marcus & Sekellick, 1975). The plaque-forming particle activity of NDV was lost at an exponential rate with the 37% survival dose ($D_{37}$ = 1 lethal hit per virion) measured as 4.25 J/m² (Marcus & Carver, 1965; Marcus & Sekellick, 1975).

Inactivation of virus by heat. NDV-AV was heat-inactivated by exposure to 55°C in a circulating-water bath. Initially, an aliquot of non-purified virus stock was diluted into prewarmed attachment solution contained in a glass tube. Samples were removed at appropriate time intervals and chilled rapidly by placing them in a salted-ice bath (Marcus & Sekellick, 1980).

Interferon induction and assay. Details of our standard procedures for interferon induction and assay have been described previously (Marcus & Sekellick, 1977, 1980; Fuller & Marcus, 1980; Winship & Marcus, 1980; Marcus et al., 1981), with several modifications: where appropriate, following virus attachment, pre-adsorbed rabbit anti-NDV serum was added at a final dilution of 1:100 to the otherwise serum-free medium bathing the cells during the induction period. This procedure prevented cycling of infection during the course of induction in permissive, 'aged' primary chick embryo cells. During interferon induction experiments in mouse L(Y) cells, inclusion of antiserum was not necessary (nor did it have any effect), due to the semi-permissive nature of this cell line to infection by wild-type NDV (Thacore & Youngner, 1970). Both mouse and chick interferon were assayed using a cytopathic effect inhibition assay (Rubinstein et al., 1981). Microwell trays containing 3 x $10^4$ mouse L(Y) cells were used 24 h after plating, for assay of mouse interferon. Primary chick embryo cells (2.5 x $10^6$ cells/well) were aged 4 days in vitro before use (M. J. Sekellick & P. I. Marcus, unpublished observations). Microwell tray covers were raised 0.5 cm on silicone rubber spacers throughout the ageing and assay portion of the experiment to maximize circulation and gas exchange. Serial dilutions of interferon were added in duplicate to microwells, and...
Did not hallucinate.
a broader peak of IFN was produced in the range $m_{\text{PFP}} = 1$ to 6. This second peak of IFN production was followed by a constant decline in yield at values of $m_{\text{PFP}} \geq 6$, whether anti-NDV serum was present or not. In the absence of anti-NDV serum, large spreading foci of haemadsorption-positive areas developed in monolayers of ‘aged’ primary CEC infected at low $m_{\text{PFP}}$, indicating cycling infection and a possible explanation for the complex nature of the dose-response curve. A simpler type of dose-response curve was observed when anti-NDV serum was added to the medium within the latent period of new virus production. At $m_{\text{PFP}} \leq 2$ the data showed a good fit to an $r > 1$ type dose-response curve (Marcus, 1982). Under these conditions, each cell infected with one, or more than one, IFN-inducing particle is presumed to respond by producing a quantum (finite) yield of IFN (Marcus, 1981/82, 1982). We can calculate the number of IFP in the NDV stock by determining the ratio of IFP : PFP at that point on the dose-response curve which represents 63% of the maximum yield of IFN. At this point there is an average of 1 IFP/cell, assuming a Poisson distribution of virus particles amongst the cell population (Marcus, 1959a, 1982). In this experiment the ratio IFP : PFP = 7, i.e. the NDV-AV stock contains seven times more IFP than PFP. [The generally accepted ratio of physical particles to PFP for NDV is about 10:1 (Isaacs, 1957). Consequently, the number of non-infectious IFP approaches the number of presumptive physical particles.] Since the PFP titre of this preparation was $6 \times 10^5$/ml, the stock contained $4.2 \times 10^9$ IFP/ml. This value (and ratio IFP : PFP) was characteristic of stocks of NDV when tested in ‘aged’ chick embryo cells. We note that the deviation from a perfect $r > 1$ type dose-response curve (Marcus, 1982) which begins at $m_{\text{PFP}} \approx 2$ represents the same conditions under which cell-killing particle (CKP) activity of NDV becomes less efficient, an effect attributed in part to a lowered probability of infection, brought about by enhanced elution of input virions due to elevated levels of virion-associated neuraminidase (Marcus, 1959a, b; Baluda, 1959).

**Mouse L(Y) cells**

Fig. 2 illustrates the results of a typical IFN induction dose-response curve generated by NDV-AV in mouse L(Y) cells, a relatively non-permissive host. The inclusion of anti-NDV serum in the growth medium did not alter the characteristics of the dose-response curve (data not shown). This result was expected, since NDV-infected L cells do not produce or release infectious virus, thus precluding cycling infection (for review, see Fleischmann & Simon, 1974). The dose-response curve describes a very good fit to an $r > 1$ type response where each cell
Survival curves of IFP and PFP exposed to u.v. radiation (254 nm) or heat (55 °C)

IFP survival measured in ‘aged’ chick embryo cells

Fig. 3 illustrates the survival of IFP activity of NDV-AV determined by infecting ‘aged’ primary CEC at a single low multiplicity \( [m_{\text{IFP}} = 1, m_{\text{PFP}} = 0.15 \text{ (before irradiation)}] \) of virus exposed to different doses of u.v. radiation. This procedure ensures that induction of IFN occurs in the region of the dose–response curve (Fig. 1, plus antiserum, \( m_{\text{PFP}} \leq 0.15, m_{\text{IFP}} \leq 1 \)) where the yield of IFN is essentially proportional to the fraction of cells infected with IFP. It is essential that these types of experiments be carried out in the presence of anti-NDV serum to prevent superinfection, thus maintaining equivalence between the input and actual value of \( m_{\text{IFP}} \). The u.v. survival curve for NDV PFP activity measured on GMK-Vero cells is shown for comparison \( [D_{37} = 4.25 \text{ J/m}^2 \text{ (Marcus & Carver, 1965)}] \). Note that about 85% of the IFP infected with one, or more than one, IFP produces a quantum yield of virus (Marcus, 1982). In this experiment, typical of several, there is an average of 1 IFP/cell at \( m_{\text{PFP}} \approx 3 \) (compare lower and upper abscissae). We interpret this to mean that only one-third as many particles as there are PFP actually register as an IFP, an observation similar to that of Fleischmann & Simon (1974; see their Fig. 1). From the data of Slattery et al. (1980; see their Fig. 3), an \( r = 1 \) type dose–response curve was generated by NDV on Ehrlich ascites tumour cells, and calculations revealed that only one-twentieth as many particles as there were PFP registered as IFP. This perhaps is not surprising in view of the failure of NDV ‘PFP’ to register very efficiently on mouse L(Y) or Ehrlich ascites cells.
activity in a stock of NDV-AV was inactivated at the same rate as infectivity (PFP). The remaining 15% of IFP activity was inactivated at about 1/9th the rate of infectivity as deduced by the change in slope of the biphasic survival curve.

**IFP survival measured in mouse L(Y) cells**

Fig. 4 shows a comparison of u.v. radiation survival curves for NDV-AV PFP and IFP activity, where the latter was determined by infecting mouse L(Y) cells with a single low multiplicity of NDV ($m_{PFP} = 0.63$; $m_{IFP} = 0.21$) which had been exposed to different doses of u.v. radiation. At low values of $m_{IFP}$ the amount of IFN produced is essentially proportional to the fraction of cells infected with IFP (see Fig. 2). At high multiplicities of irradiated NDV, and the apparent size of the u.v. target for IFP activity decreases (data not shown), suggesting some multiplicity-dependent rescue of virus function (compare Peeples & Bratt, 1982). Again, as in Fig. 3, PFP activity was assayed on GMK-Vero cells. Both PFP and IFP activities are lost at essentially the same exponential rate, describing a value for $D_{37} = 4.25$ J/m².

**Survival curves of NDV PFP and IFP exposed to heat (55 °C)**

Fig. 5 demonstrates that both PFP and IFP activities [as measured on mouse L(Y) cells at low $m_{IFP}$] are lost at the same rate when heated at 55 °C. The exponential character of the survival curve makes it amenable to analysis by the expression $S = e^{-kt}$ where $S$ is the surviving fraction of activity after time $t$, and $K$ is an inactivation constant. From this curve, the rate of inactivation of NDV-AV at 55 °C is calculated as $K_{NDV} = 0.15$/min. Similar results were obtained with 'aged' CEC as hosts (data not shown). These results confirm and extend the observations of Youngner et al. (1966), Kohno et al. (1969), and Sheaff et al. (1972), and indicate that PFP and IFP share a similar rate-limiting step for expression. From these studies we conclude that the heat-sensitive step represents inactivation of NDV virion-associated transcriptase, and, hence, that transcription of the NDV genome is required for both PFP and IFP activity.

**NDV PFP are phenotypically ifp⁻ on chick embryo cells**

When Sindbis virus is assayed for plaque formation on chick embryo cells both the efficiency of plaquing and the size of plaques decrease markedly with 'age' of the cell monolayer (Carver & Marcus, 1967). This characteristic was attributed to the ifp⁺ (capable of inducing IFN) phenotype of Sindbis virus and its extreme sensitivity to IFN action. In contrast, the plaque size of NDV on either 'unaged' or 'aged' primary CEC is not different, and continues to increase with time of incubation at 37.5 °C or 40.5 °C irrespective of plaque density (data not shown; Lockart, 1968). Thus, according to the observations of Francoeur et al. (1980), NDV functions as a PIF⁻ (plaque interferon-negative) virus. We note, however, that this phenotype would also be
expressed by a virus which induced IFN, but which was resistant to its action. We observed that 1 or 2 PR50 (NDV) units of IFN reduced the plaque size of NDV significantly (from 1 mm to ≤0.5 mm). This provides evidence that NDV would be sensitive to IFN if induction had occurred. On that basis we conclude that the PIF- character of NDV PFP on CEC is due solely to its ifp- phenotype.

The IFN-inducing capacity of u.v.-irradiated NDV: a theoretical appraisal and model

Active NDV induces little or no IFN in monolayers of conventional 'unaged' chick embryo cells, and hence can be described phenotypically as ifp-. However, upon exposure to low doses of u.v. radiation (or upon inactivation by other agents) NDV stocks rapidly acquire an ifp+ phenotype (for review, see Johnston & Burke, 1973; C. Svitlik & P. I. Marcus, unpublished observations). This phenotype is eventually lost upon exposing the virus to higher doses of u.v. radiation, and the population gradually reverts to ifp- status, with the IFN-inducing capacity decaying exponentially in the high dose range. There is a striking uniformity in the data which describe IFN induction in 'unaged' chick embryo cells by NDV exposed to different doses of u.v. radiation (Ho & Breinig, 1965; Youngner et al., 1966; Kohno & Kohase, 1969; Gandhi et al., 1970; Clavell & Bratt, 1971; Meager & Burke, 1972; Kowal & Youngner, 1978). We interpreted these curves in terms of viral functions that might affect expression of IFP activity, and by postulating a set of u.v.-sensitive targets in the viral genome controlling these functions. These targets are represented schematically in Fig. 6 and form the basis of a model for generating IFP from NDV with u.v. irradiation. All targets, regions A to D, share a border at the 3' terminus of the genome, where transcription is initiated through a single promoter sequence (T). A u.v. hit [uracil dimer formation: Miller & Plagemann (1974); Collins et al. (1980)] to a target prevents transcription downstream. The regions are defined as follows (Fig. 6).

Region A = IFP- (4% target). A hit here inactivates IFP capacity.
Region B = IFP* (5% target). Potential IFP.
Region B-crosshatch = IFP (5%-4% = 1% target). A hit here confers IFP status.
Region D = PFP (100% target). The entire genome. A hit here inactivates infectivity.

The most novel feature of this model is that generation of an IFN-inducing particle results from a single hit to a specific region in the NDV genome, located between 4% and 5% of the distance from the single initiation site for transcription at the 3' terminus. This critical target is designated B-crosshatch in the diagram (Fig. 6). There is a low probability of inactivating the function of this target upon u.v. irradiation because of the small size of B-crosshatch (1%), and its location near the 3' terminus of the genome. Whereas this region must sustain a single hit to confer IFP status, region A (4% target) must remain undamaged. Because of its location at the initiation site for transcription at the 3' terminus (Collins et al., 1980), region A is not inactivated by u.v. hits acquired anywhere else in the genome, i.e. the remaining 5' distal 96%, represented by regions B-crosshatch (included within B, C and D), C (included within D), and D. Presumably, a u.v. hit in the B-crosshatch region will prevent transcription through this region and allow for double-stranded (ds)RNA formation between the RNA template represented by region A (4% target) and its transcript.

For the reasons cited, high doses of u.v. radiation are required to hit the small, 1% target (B-crosshatch) and confer IFP status. At even higher u.v. doses, IFP activity is lost at a constant exponential rate representing hits in the 4% target (region A) as defined by the average slope of inactivation observed experimentally (Collins et al., 1980; Fig. 7b).

We also assume that most, or all, non-infectious IFP in NDV stocks are not functional in conventional 'unaged' CEC. Infectious particles are phenotypically pfp+, isp+, ifp-, i.e. they do
Fig. 7. (a) Distribution curve representing the fraction of phenotypically $\text{ifp}^+$ NDV ($= \text{IFP}$) generated from a population of otherwise phenotypically $\text{ifp}^-$ NDV ($= \text{PFP}$) upon u.v. irradiation, in accord with the following assumptions. NDV IFP are presumed to be generated from phenotypically $\text{ifp}^-$ virus when 96% of the 5' proximal portion of the genome has sustained damage from u.v. radiation, while the 4% (region A in Fig. 6) of the genome nearest the 3' end survives undamaged and remains transcribable. The ordinate represents, normalizing to maximum, the fraction of IFP activity calculated from a Poisson distribution for the class of virus particles which satisfies these conditions. Thus, $P_{\text{IFP}} = 1 - e^{-h}$, where $h$ is the average number of u.v. hits to the entire genome (region D). For NDV, $h = 4.25 \text{ J/m}^2$ (Marcus & Carver, 1965). The average number of hits to a 5% target (region B) in a genome exposed to $h$ hits is given by the term $1 - e^{-0.05h}$. Similarly, the average number of hits to a 4% target (region A) is represented by $1 - e^{-0.04h}$. Generation of an IFP is postulated to occur when a hit is introduced into a special 1% region (B-crosshatch) of the genome. (Note that region A remains unhit.) The probability of this event occurring is represented by the following term:

$$P_{\text{IFP}} = (1 - e^{-0.05h}) - (1 - e^{-0.04h})$$

This function defines a region that lies between 4% and 5% of the genome (B-crosshatch) that must acquire a u.v. hit to convert a phenotypically $\text{ifp}^-$ particle into a functional IFP while maintaining the 3' most-proximal 4% of the genome (region A) intact and transcribable. (b) Comparison of observed data (four sets of experimental points) and a theoretical curve (solid line) for the yield of IFN produced by 'unaged' CEC infected by u.v.-irradiated NDV, as a function of u.v. dose. The data points represent experiments from four different publications [Youngner et al., 1966 (●); Kohno & Kohase, 1969 (□); Meager & Burke, 1972 (□)]; and Kowal & Youngner, 1978 (●)] normalized to maximum IFN yield. The solid line represents, as a function of u.v. dose, the yield of IFN (normalized to maximum) predicted from a cell population infected with the virus population illustrated in (a). To attain this fit to the observed data the following assumptions were made. (i) Each cell infected with 1 or more IFP, and only IFP, produced a quantum yield of IFN (Marcus, 1982), (ii) each PFP also expressed ISP activity, i.e. ISP : PFP = 1, (iii) a cell co-infected with an IFP plus 1 or more ISP does not yield IFN, (iv) to express ISP activity about 16% of the T-proximal region of the NDV genome (NP gene?) must remain undamaged by u.v. radiation, and (v) in the example illustrated (solid line), $m_{\text{IFP}} = 5$ (before irradiation). Under these conditions, and as a function of u.v. dose, the fraction of maximum yield of IFN can be determined where:

$$P_{\text{IFN yield}} = (1 - e^{-xm}) \times (e^{-mc^{-0.16h}})$$

Here, $m$ is the multiplicity of infection (before irradiation) and $x$ is the fraction of NDV IFP calculated in (a) as $P_{\text{IFP}}$. The term $(1 - e^{-xm})$ represents the fraction of the cell population infected with IFP for a given u.v. dose. The fraction of the cell population that escapes infection with ISP is represented by the term $(e^{-mc^{-0.16h}})$ where 0.16 is the number of u.v. hits to the 16% target required for ISP expression. The complete function, the product of these two terms, expresses the probability of an IFP infecting a cell lacking an ISP, and hence the fraction of IFN-producing cells for a given dose of u.v. radiation. Under the conditions illustrated in (b), where $m = 5$, the maximum fraction of IFN-producing cells, before normalization, is 0.31; this was achieved at a dose of about 130 J/m². This dose delivers about 31 u.v. hits to the whole genome (PFP = 100%, target D), 4-9 hits to the ISP (16%, target C), 1-5 hits to the IFP* (= potential IFP) region (5%, target B) and 1-2 hits to the IFP - region (4%, target A). A hit in the 'window' between the 4% and 5% targets, region B-crosshatch, renders the particle IFP.
not induce IFN and dominantly suppress IFN induction in a cell co-infected with IFP. However, PFP are potential IFP, and upon u.v. irradiation can be converted to the phenotype pf +, isp −, ifp +, i.e. they become capable of inducing IFN.

Based on these targets and a Poisson distribution of u.v. hits to the NDV genome a theoretical curve (Fig. 7a) was calculated to represent the fraction of IFP generated as a function of u.v. dose, normalizing the maximum value to 1. For these calculations D25 = 4.25 J/m² for NDV PFP (Marcus & Carver, 1965; Marcus & Sekellick, 1975), setting the D25 values for 5% (region B) and 4% (region A) u.v. targets in the NDV genome at 85 and 106.3 J/m², respectively. As illustrated in Fig. 7(a), the fraction of NDV IFP generated as a function of u.v. dose is extremely low initially, it increases rapidly in the low dose region [this presumably reflects the acquisition by the virus population of hits in the 1% (5% - 4%) ‘window’ (B-crosshatch) at the 3’-proximal region of the genome] and reaches a plateau at about 100 J/m² (≈24 lethal hits to PFP; equivalent to an average of 1 hit to region B-crosshatch). The absolute value of the fraction of IFP in the NDV population at the peak yield is 8.1% before normalization. This low value is consistent with the relatively low yields of IFN reported from ‘unaged’ CEC infected with irradiated NDV.

In Fig. 7(b) the yield of IFN expected (solid line) when a population of irradiated NDV with the characteristics shown in Fig. 7(a) is used to infect cells, is compared with the actual yield of IFN reported in four different studies (data points). Note that, in order to obtain a good fit to the observed data, it was necessary to postulate an additional attribute of NDV, one that acts to suppress IFN induction. This function is relatively resistant to u.v. radiation and is postulated to require expression of about the 16% of the genome nearest the 3’ end (region C, NP gene?). This function confers ISP activity on the particle, and cells infected with an IFP plus one or more ISP fail to produce IFN, i.e. are phenotypically ifp −. The D25 value for this target is 26.6 J/m². [This attribute is in keeping with the concept advanced by Kohno et al. (1969) and elaborated by Meager & Burke (1972), that the failure of infectious virus to induce IFN may be due to the production of a viral protein that inhibits IFN formation. Reconstruction experiments (Sheaff et al., 1972) support the view of dominance of this protein’s activity, an effect we have confirmed and which is accommodated in our model as the dominant expression of ISP over IFP activity (see Marcus, 1982).] The theoretical curve generated in Fig. 7(b) represents the IFN yields expected if the cells were infected at $m_{u,v,PFP} = 5$. The fit improves at lower multiplicities of infection, and is near perfect at $m_{u,v,PFP} = 1$. In this context we note that the actual $m_{u,v,PFP}$ may be significantly lower than the input $m_{u,v,PFP}$ because of disproportionately high rates of virus elution at high $m$ values of input irradiated NDV (Baluda, 1959; Marcus, 1959a, b).

**DISCUSSION**

In a series of earlier studies we analysed the IFN induction dose–response curves of infectious virus, defective virus particles, mutant viruses, and virions inactivated with u.v. radiation and heat (for reviews, see Marcus, 1981/82, 1982). An approach was developed which permitted, through statistical inference, the detection and quantification of virus particles in terms of their IFN-inducing particle activity. We established that one IFP per cell sufficed to induce in that cell a quantum yield of IFN. Using this approach, the present study revealed that stocks of wild-type NDV-AV contained, in addition to infectious virus (PFP), a large excess of non-infectious IFN-inducing particles (IFP). The ratio of IFP : PFP was about 7. These IFP were detected in ‘aged’ primary CEC, but not on mouse L cells, thus qualifying them as host-dependent IFP. Since these host-dependent IFP were non-infectious, their presence in stocks of NDV was detected only by analysing the ratio of IFN induction curve they generated (Marcus, 1982), and finding that IFN was being induced at multiplicities of NDV well below that expected were PFP solely responsible for the observed yield of IFN.

On mouse L(Y) cells, stocks of NDV-AV generated an $r \geq 1$ type of IFN induction dose–response curve displaying a ratio of IFP : PFP about 0.3, suggesting only about one-third as many particles as PFP scored as IFP. This ratio cannot be attributed to incomplete proteolytic cleavage of the fusion glycoprotein (F), since virtually all of F present in virions of NDV-AV is in the cleaved state (Smith & Hightower, 1981). It is also clear from the studies of Ito et al. (1982)
Table 1. Interferon-inducing and plaque-forming particle phenotypes of Newcastle disease virus on different host cells

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>U. v. dose</th>
<th>'Unaged' CEC</th>
<th>'Aged' CEC</th>
<th>Mouse L</th>
</tr>
</thead>
<tbody>
<tr>
<td>ifp⁻, pfp⁻*</td>
<td>None</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ifp⁺, pfp⁻†</td>
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<td>-</td>
<td>+</td>
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<tr>
<td>ifp⁻, pfp⁻§</td>
<td>High</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Host-dependent IFP.
† Host-dependent non-infectious IFP.
‡ IFP created by low doses of u.v. radiation to PFP.
§ Loss of IFP due to damage in the region of the genome essential for IFP expression (see Fig. 6).

that in mouse L cells proper proteolytic cleavage of the F protein is required for IFN induction by NDV. The ratio 1 IFP : 3 PFP does not appear to be due to the activity of ISP in mouse L(Y) cells, since the dose–response curve does not display a negative slope at high values of m<sub>PFP</sub>.

Many variables characterize IFN induction by NDV and regulate the ifp phenotype intrinsic to the virus. Table 1 summarizes these variable and shows how they are altered by u.v. radiation and the use of different host cells. The complications associated with IFN induction by NDV become apparent. It appears that relative to PFP, only one-third as many IFP register in mouse L cells, a finding consistent with observations of Fleischmann & Simon (1974). This compares with only one-twentieth the number of PFP registering as IFP on Ehrlich ascites tumour cells (Slattery et al., 1980). In contrast, 'aged' chick embryo cells appear ideal hosts for NDV IFP activity, since they can be used for scoring a new class of inducers, particles which are non-infectious. These particles do not plaque on GMK-Vero cells even though IFN cannot be induced on these cells. Hence, feedback inhibition of virus replication through IFN action (Francoeur et al., 1980) cannot account for the failure of these IFP to function as infectious particles.

The model developed to explain the ifp⁻ → ifp⁺ → ifp⁻ status achieved by NDV on 'unaged' chick embryo cells, with increasing doses of u.v. light, appears to account satisfactorily for the IFN-inducing capacity of this virus. This is characterized by myriad induction responses on different host cells as the virus is progressively inactivated by u.v. light, heat or chemical reagents. The model identifies a portion of the NDV genome towards the 3' end which must remain intact and transcribable to express IFP activity. Based on u.v. target data from several studies, a mean value for this target appears to be about 4% of the genome. In the extremes, some studies show u.v. targets of from 2% to 10%. Based on a model for the formation of the IFN-inducing moiety in cells infected with VSV, another negative-strand virus phenotypically ifp⁺ (Marcus & Sekellick, 1980), we postulate that transcription of the 3'-proximal 4% of the NDV genome leads eventually to extensive base-pairing between genome RNA and its complementary transcript. We suggest that the dsRNA thus formed constitutes the IFN inducing moiety of NDV. This form of the inducer was first suggested by Huppert et al. (1969), and has gained credence with further experimentation (Clavell & Bratt, 1971; Sheaff et al., 1972). Indeed, the experiments of Dianzani et al. (1970), carried out before the discovery of NDV-associated transcriptase, actually demonstrated that in the presence of a double-block to protein synthesis NDV could form an IFN inducer molecule(s), and that the induction process itself, leading to gene transcription, could take place.

Apparently, expression of this 4% of the genome (region A in Fig. 6) does not suffice to induce IFN; rather, a u.v. hit in a slightly more distal region, designated B-crosshatch, seems essential. This leads to the concept of a critical region in the genome adjacent to, and extending beyond, the 4% target by about 1% which must be hit to convert virions from an ifp⁻ to an ifp⁺ phenotype. It is not clear what this means in molecular terms. Conceivably, transcription beyond the 4% region results in disassociation of a partial NP (nucleoprotein: the first 3'-proximal gene) transcript from its template, thus aborting formation of a putative IFN inducer molecule, dsRNA. A u.v. hit in the critical 1% target (region B-crosshatch) required for creating
an IFP may occur at a special site in the RNA genome. When transcription through this site is blocked by uracil dimer formation, base-pairing between the RNA template and the transcript synthesized up to that point may be facilitated. [A special site may exist in the comparable N gene of VSV (Singh et al., 1982).]

Finally, to fit a model for induction to the observed data it was necessary to introduce the notion of a 16% target (region C) at the 3'-proximal end of the NDV genome whose expression results in the suppression of IFN induction in any cell co-infected with a particle expressing this region and an IFP. This condition permits calculation of a theoretical fraction of IFN-producing cells at a rate comparable to that actually observed when 'unaged' chick embryo cells are infected with u.v.-inactivated NDV over a relatively broad range of u.v. PFP. A model in which inactivation of infectivity (1 u.v. hit to the NDV genome) also inactivates the ISP function, as suggested by Youngner et al. (1966), is precluded since it would result in too rapid a rate of increase in the fraction of IFN-producing cells as a function of u.v. dose. However, a 16% region for ISP expression allows for a good fit between actual and expected results. We note with interest, but without understanding its significance, that a '16 %' target essentially represents the size of the NP gene, the first gene transcribed in infection, and that nearest the 3' terminus (Collins et al., 1980, 1982). Conceivably, new NP acts to inhibit viral dsRNA formation by binding to newly formed transcripts. Alternatively, NP might have a deleterious effect on the expression of cellular genes (see Marvaldi et al., 1978; Jaye et al., 1982). In any event, the reconstruction experiment of Sheaff et al. (1972) shows that the ISP character is dominant to the expression of IFP (see Marcus, 1982).

Using 'unaged' CEC as hosts for IFN induction we found that high doses of u.v. radiation were required to generate IFP from otherwise ifp- NDV PFP in a manner similar to that shown in Fig. 7(b) (data not shown). We presume that these particles also register in 'aged' CEC and contribute to the total yield of IFN produced over a broad range of u.v. doses. However, when 'aged' CEC are used as hosts (Fig. 3), it is apparent that the generation of IFP from PFP required much lower doses of u.v. radiation, suggesting that a hit to a much larger region of the genome sufficed to create IFP activity (deduced from the u.v. survival curve in Fig. 3). All of the IFN induced by unirradiated NDV in 'aged' CEC can be attributed to the activity of non-infectious IFP which are inactivated by a single u.v. hit to the genome. We postulate that low doses of u.v. radiation may serve to inactivate all non-infectious IFP intrinsic to the stock, and generate new IFP from PFP. The concerted expression of these two classes of IFP may explain the biphasic nature of the u.v. survival curve observed experimentally (Fig. 3).

Obviously 'unaged' and 'aged' CEC are functionally very different hosts with respect to the expression of IFP activity by u.v.-irradiated NDV. Note though, in either cell state there remains the requirement for transcription of only a fraction of the NDV genome to induce IFN.

Consider now the non-infectious IFP detectable only on 'aged' CEC; the requirement for expression of the entire genome may reflect the need for new transcriptase to produce sufficient rounds of transcription to ensure dsRNA formation. In this context, we note CEC infected with non-cytopathic mutants of NDV-AV synthesize lower levels of virus-specific RNA (Madansky & Bratt, 1981a) and protein (Madansky & Bratt, 1981b) than parental or revertant virus. A decrease in the rate of RNA synthesis might lower the probability of forming dsRNA, whereas amplified RNA synthesis might increase it. In this context a deficiency of NP might favour base-pairing between template and transcript, as we have previously suggested as the basis for dsRNA formation in another negative-strand virus, VSV (Marcus & Sekellick, 1980).

Clearly, NDV appears to be one of the most complex viral inducers of IFN thus far studied. The model presented here offers some insight into the processes whereby IFN induction may be regulated through the differential expression of genes in the inducing virus.

This study was supported by research grants from the National Institute for Allergy and Infectious Diseases (AI 18381) and the National Science Foundation (PCM 80-21882), and benefited from the use of the Cell Culture Facility supported by National Cancer Institute grant CA 14733. Some aspects of this paper were presented at the 2nd Annual Meeting of the American Society for Virology at Michigan State University, East Lansing, Michigan, U.S.A. on July 13, 1983. We thank Dr. Uwe Koehn of the Department of Statistics for confirming by computer analyses the theoretical curves generated in Fig. 7, based on the model presented.
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(Received 24 March 1983)