Inhibition of Early Vaccinia Virus Protein Synthesis in Interferon-treated Chicken Embryo Fibroblasts

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SUMMARY

The effect of interferon pre-treatment on early virus protein and RNA synthesis has been examined in chicken cells infected with vaccinia virus. Protein synthesis was inhibited while RNA synthesis was stimulated.

There exists some controversy concerning the mechanism by which interferon treatment inhibits virus multiplication. We have recently demonstrated that, in L cells infected with vaccinia virus, early virus protein synthesis is inhibited when the cells are pre-treated with interferon, while the synthesis of virus RNA by the particle RNA polymerase is not (Metz & Esteban, 1972). This result is consistent with the earlier observations of Joklik & Merigan (1966), but not with the recent report of Bialy & Colby (1972), who claimed that interferon pre-treatment of chicken embryo cells inhibited the activity of the polymerase in the infected cell. Since the latter authors had employed chicken cells and since Marcus et al. (1971) had found that the activity of the particle polymerase of vesicular stomatitis virus was inhibited in interferon-treated chicken cells, it seemed possible that the mechanism of action of interferon might be different in the line of mouse L cells and in primary or secondary chicken embryo fibroblasts. Consequently, we examined the sensitivity of early virus RNA and protein synthesis to interferon treatment in vaccinia-infected chick embryo cells. We report that the situation is very similar to that which we had found earlier for L cells.

The growth and purification of vaccinia virus strain WR, the growth of L cells, the source of purified mouse interferon, the infection of cells in suspension, the labelling of cells in suspension with [3S]-methionine, the procedure for polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate and autoradiography, and the measurement of the rate of cytoplasmic RNA synthesis have all been described (Metz & Esteban, 1972; Esteban & Metz, 1973). Chick embryo cells were used either as monolayers or in suspension. In the latter case 48 h monolayers were treated briefly with trypsin and resuspended in Eagle’s medium (spinner modified), buffered with tris at pH 7.2 (measured at 25 °C) containing 5% calf serum, at a concentration of 1.5 x 10^6 cells/ml. After stirring for 2 h at 37 °C, they were infected with vaccinia virus at an input multiplicity of 500 particles (about 5 p.f.u./cell) as described. Infection of monolayers of chick or L cells (35 mm dishes, 4 to 5 x 10^6 cells) employed an input multiplicity of 2000 particles/cell in a vol. of 0.1 ml of Puck’s saline A + 20 mm-MgCl_2 for 15 min at 37 °C. The dishes were then rapidly washed with Eagle’s medium (tris buffered, plus 5% calf serum) and then incubated with 2 ml medium, this being designated ‘zero time’. For labelling with [35S]-methionine, monolayers were washed with Eagle’s medium (lacking methionine, buffered with tris), and then incubated with 0-5 ml of this medium plus 5 μCi [35S]-methionine (about 20 Ci/m-mol) for 20 min. Monolayers were chilled, washed with cold buffered saline and extracted at 100 °C with sodium dodecyl sulphate–urea–mercaptoethanol mixture. Chick cell monolayers at 24 h were treated with chick interferon (2-0 per 35 mm dish or 15 ml per 135 mm dish) for 16 h at 37 °C prior to infection in monolayer or to preparation of suspension cultures for subsequence.
sequent infection. Partially purified chick interferon was generously provided by Dr K. Fantes (Fantes, 1967). Most experiments were done using a single batch, which, when prepared, had a specific activity of 30 000 units/mg protein. It had been stored for a number of years at −70 °C and was re-assayed prior to use. The activity had decreased by about twofold. Some experiments employed chick interferon with specific activity of 300 000 units/mg. Identical results were obtained with the two preparations. Activities are expressed in terms of units defined by the MRC Research Standard A 62/4 for chick interferon. One such unit was found to reduce the yield of vaccinia virus in chick embryo cells by 50% under the conditions employed in the present experiments.

We have recently described the pattern and time course of vaccinia polypeptide synthesis in infected L cells (Esteban & Metz, 1973). The pattern of [35S]-methionine labelled polypeptides derived from extracts of infected chick cells is quite distinct from that from uninfected cells (Fig. 1a). This is the case when chick cells are infected in monolayer or in suspension, though in the former case there is less cut-off of cellular protein synthesis and hence polypeptides common to infected and uninfected cells are more numerous (not shown). The new polypeptides seen in extracts of infected chick cells are very similar in electrophoretic mobility to those from infected L cells (Fig. 1a). Furthermore, when virus RNA synthesis in infected chick cells is inhibited by the drug cordycepin no new polypeptides are detected. We have previously argued that the new polypeptides seen in vaccinia-infected L cells are virus-coded. The same arguments apply to the case of vaccinia-infected chick cells. The fact that the patterns of new polypeptides seen following infection of both L and chick cells are very similar is an additional item of evidence for their being specified by the virus.

The effect of pre-treatment with different doses of chick interferon on polypeptide synthesis in infected chick cell monolayers is shown in Fig. 1(b). The synthesis of the new polypeptides is clearly sensitive to interferon. Ten units/ml of interferon is sufficient to reduce very substantially the synthesis of virus polypeptides as early as 20 min after infection. The synthesis of polypeptides which are present in both infected and uninfected cells, and which are therefore presumably cellular, is also inhibited by interferon. Detailed analysis of the polysome patterns from interferon-treated, vaccinia-infected L cells has indicated that a similar situation applies there and that it may be a consequence of the enhancement of virus RNA synthesis which occurs in both systems (D. H. Metz & M. Esteban, unpublished observations).

Inhibition of the synthesis of virus polypeptides could be due to inhibition of the translation of virus messenger RNA or to the inhibition of the synthesis of this RNA. We have therefore examined the rate of RNA synthesis in vaccinia-infected chick cells under a variety of conditions. There is a burst of RNA synthesis reaching a peak at about 30 min after infection (Fig. 2). The RNA synthesis is sensitive to actinomycin D. In the presence of cycloheximide or following interferon pre-treatment, RNA synthesis is prolonged. Treatment with both interferon and cycloheximide results in a rate of RNA synthesis very similar to that found with one or other treatment alone (not shown). This pattern of RNA synthesis in infected chick cells is quite similar to that found in L cells (Metz & Esteban, 1972). In both cases, inhibition of protein synthesis by cycloheximide results in a stimulation of RNA synthesis. The simplest interpretation of this phenomenon is that the switch-off of virus RNA synthesis is mediated by a newly synthesized virus protein which is coded by this same RNA. In chick cells virus RNA synthesis is sensitive to actinomycin D. In this respect chick cells resemble HeLa cells rather than L cells (Moss, 1968; Metz & Esteban, 1972). We have previously suggested that the degree of sensitivity to this inhibitor depends upon the
Fig. 1. (a) Polyacrylamide gel analysis of [35S]-methionine-labelled polypeptides extracted from vaccinia-infected and uninfected suspensions of chick and L cells. Cells were labelled at 20 min after infection for 20 min. Cu, uninfected chick cells; Ci, infected chick cells; Lu, uninfected L cells; Li, infected L cells.

(b) Polyacrylamide gel analysis of [35S]-methionine-labelled polypeptides extracted from interferon-treated, vaccinia-infected suspensions of chick cells. Cells were treated with 0, 10, 40 or 80 units/ml of chick interferon in monolayer, as indicated, prior to infection in suspension. Cells were labelled for 20 min, at 20 or 60 min after infection (left and right, respectively). U indicates the pattern for uninfected cells.
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Fig. 2. The rate of RNA synthesis in vaccinia-infected chick cells in suspension. The counts shown are a measure of the incorporation into acid-insoluble, cytoplasmic extracts prepared from samples of cells labelled with [H]-uridine for 10 min at different times after infection. There is a 15 min absorption period prior to 'zero time'. □—□, uninfected cells; ●—●, infected cells; △—△, cells infected in the presence of 100 µg/ml cycloheximide; ○—○, cells infected following pretreatment with 40 units/ml of interferon; ■—■, cells infected in the presence of 1 µg/ml actinomycin D.

permeability of the core structure which, in turn, may depend upon the cell-mediated first stage of uncoating.

The effect of interferon pre-treatment results in a stimulation of virus RNA synthesis in vaccinia-infected chick cells just as it does in L cells. Jungwirth et al. (1972) have reported similar results for vaccinia in both chick and L cells and have further characterized the RNA as virus specific by hybridization to virus DNA. It is evident that the inhibition of virus protein synthesis effected by interferon pre-treatment of the cells is not due to inhibition of virus messenger RNA synthesis.

It seems clear from these results that there is no significant difference in the mechanism of action of the interferon-mediated inhibition of vaccinia virus replication in primary chick embryo cells and in a line of mouse L cells. Our results are in marked contrast to those of Bialy & Colby (1972), who concluded that interferon treatment blocked the transcription of virus RNA. We have discussed previously possible explanations of this discrepancy (Metz & Esteban, 1972). It may be stressed that there is no especial cytotoxic effect found in interferon-treated, vaccinia-infected chick cells (Jungwirth et al. 1972) comparable to that found in L cells (Joklik & Merigan, 1966; Horak, Jungwirth & Bodo, 1971). Hence, suggestions that the stimulation of RNA synthesis observed in interferon-treated, infected cells may be connected with this cytotoxicity seem untenable.

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