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INTERFERON-INDUCED SYNTHESIS OF A 63,000 DALTON PROTEIN IN MOUSE CELLS

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Summary : Cultures of mouse JLSV5 cells (a cell line chronically infected with Rauscher murine leukemia virus) and of fresh uninfected NMRI mouse embryo fibroblasts were treated with interferon and labelled with (^{35}S)-methionine. Newly synthesized proteins were then examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and two-dimensional electrophoresis of the cell lysates. In both cell types interferon treatment resulted in the synthesis of a radioactive 63,000 dalton protein, which was undetectable in a radioactive form in the control cells. The possibility is considered that this protein is a mediator of the biological activities of interferon on cells.

The exposure of cells to homologous interferon results in broad antiviral resistance. The development of this resistance can be prevented by treatment of the cells with inhibitors of transcription (e.g. actinomycin D) or translation (e.g. cycloheximide). From this it has been inferred that interferon acts by induction of one or more so-called "antiviral proteins" (1,2). In order to detect such proteins many investigators have analysed cell lysates for the presence of factors that could block one or more cellular functions that are essential in virus replication. Thus the following enzymatic activities have been observed in lysates of interferon-treated cells : a membrane-bound alkaline ribonuclease (3,4), an adenylate cyclase (5), a ribosome-associated nucleolytic activity (6), a protein kinase (7,8,9), a ds-RNA dependent (2'-5')A_n synthetase (9,10,11) and a phosphodiesterase (12). So far only the (2'-5')A_n synthetase and the protein kinase(s) have been partially purified and separated from each other (13,14,15). In order to help define interferon-induced proteins we have analysed lysates of interferon-treated JLSV5 cells by SDS-PAGE (16) and by 2-D-electrophoresis (17). The interferon was prepared and titrated on L-929 cells as described (18). It had a specific activity of $10^{6.5}$ U/mg.

Abbreviations : SDS-PAGE : sodium dodecyl sulfate-polyacrylamide gel electrophoresis; 2-D-electrophoresis : two-dimensional electrophoresis; ds-RNA : double-stranded RNA; EMEM : Eagle's minimum essential medium; PBS : phosphate buffered saline; EDTA : ethylenediamine tetraacetic acid; TPCK : L-1-tosylamide-2-phenylethylchloromethyl ketone; PMSF : phenylmethyl sulfonyl-fluoride; DATD : N-N'-diallyltartardiamide.

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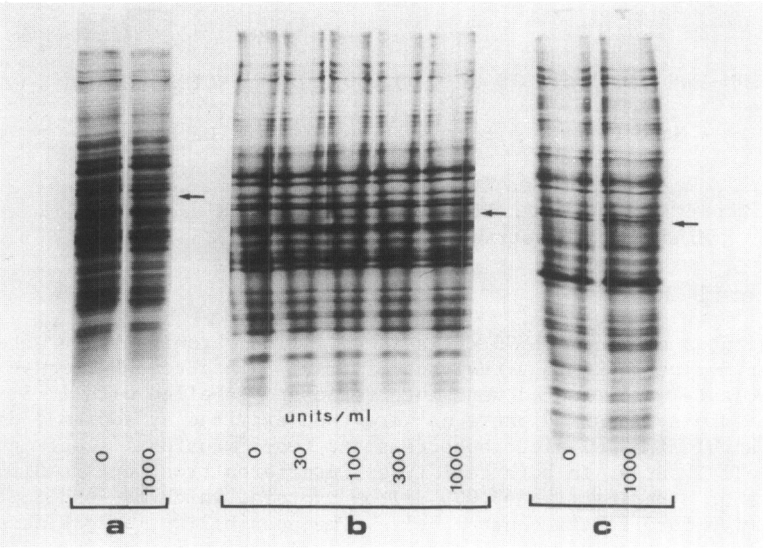

FIG. 1. SDS-PAGE of lysates from control and interferon-treated cells. Confluent cultures were incubated for 24 hours with indicated doses of interferon in the presence of $[^{35}S]$L-methionine. (a) and (b) : JLSV5 cells; (c) : mouse embryo fibroblasts. Arrows indicate position of 63,000 dalton protein.

JLSV5 cells were grown in 75 cm^2 tissue culture flasks (Falcon plastics, Oxnard, California) in EMEM supplemented with 10 % inactivated (56°C, 30 min) foetal bovine serum, 2 mM glutamine and 3 % bicarbonate, and kept at 37°C in a 5 % CO_2 atmosphere. When the cultures had reached confluency, they were washed with Dulbecco's PBS (with Ca^{2+} and Mg^{2+}), and refed with 10 ml of methionine-free EMEM supplemented with 2 % inactivated and dialyzed foetal bovine serum, 2 mM glutamine, 3 % bicarbonate and 1.5 mg/l L-methionine. After 2 hours the cultures were refed with 10 ml of the same medium, together with 0 or 10^3 U/ml of mouse interferon and 200 µCi $[^{35}S]$L-methionine (1350 Ci/mmol, The Radiochemical Centre, Amersham, Buckinghamshire, England). After a further 24 hr of incubation at 37°C the cells were washed with cold PBS and cell lysates were prepared. The cells were scraped into 1 ml of 5 mM Tris-HCl, pH 9.2, containing 1 mM EDTA, 400 mM KCl, 1 % (v/v) Triton X-100, 1 mM TPCK and 1 mM PMSF, per culture flask. After incubation for 15 min at 37°C the suspensions were centrifuged at 27,000 g for 10 min. The pellets were resuspended in 1 ml of the same buffer but without KCl, incubated for 15 min at 37°C and centrifuged. The combined supernatants were dialyzed against TNE buffer (20 mM Tris-HCl, pH 7.4, 1 mM EDTA, 100 mM NaCl). The dialyzed cell lysates were clarified at 100,000 g for 1 hr and analysed by discontinuous SDS-PAGE on a gradient gel (8-24 % polyacrylamide, DATD cross-linked) with a 3 % polyacrylamide stacking gel. Phosphorylase a (93,000 dalton), bovine serum

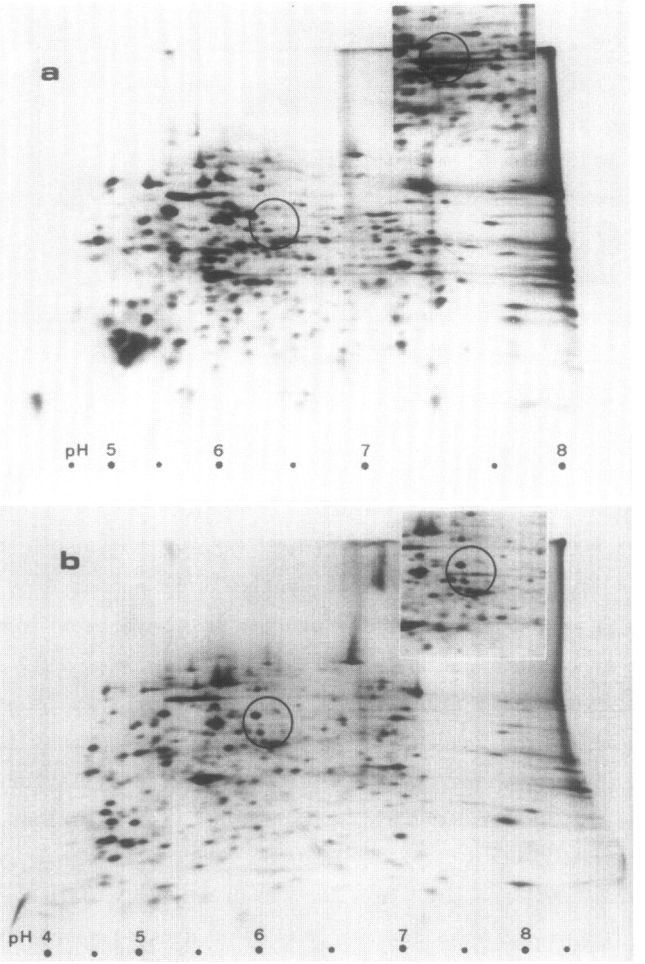

FIG. 2. 2-D-Electrophoresis of lysates from control and interferon-treated cells. Confluent monolayers were incubated for 24 hours with 0 or 1,000 units/ml of interferon in the presence of $[^{35}S]$L-methionine. (a) : JLSV5 cells; (b) : mouse embryo fibroblasts. Insets show patterns obtained with interferon-treated cells as opposed to those of control cells, shown in full.

albumin (68,000 dalton), ovalbumin (43,000 dalton), chymotrypsinogen (25,000 dalton), and lysozyme (14,000 dalton) were used as molecular weight standards. The gels were prepared for fluorography (19,20) and exposed to X-ray films (Curix RP2, Agfa-Gevaert, Mortsel, Belgium) at -70°C. Figure 1a shows the fluorographs of the SDS-PAGE of cell lysates prepared from control and interferon-treated JLSV5 cells. It can be seen that they were completely identical with the exception of one protein band at 63,000 dalton which was only detectable in the lysate of interferon-treated cells. A similar experiment using increasing doses of interferon showed that the 63,000 dalton protein was al-

ready induced by 100 U/ml (Fig. 1b). Finally, Figure 1c shows that synthesis of this protein was also induced in first passage mouse embryo fibroblasts from NMRI mice (Proefdierencentrum, K.U.Leuven, Belgium). The cell lysates were also analysed by 2-D-electrophoresis. Although the overall protein pattern of JLSV5 cells and of mouse embryo fibroblasts were quite different, interferon treatment resulted for both cell types in the induction of the 63,000 dalton protein with an isoelectric point of 6.3 (Fig. 2).

JLSV5 cells are chronically infected with Rauscher murine leukemia virus. It is known that treatment of such cells with interferon inhibits the release of virus particles and leads to accumulation of viral proteins in the cells (21). The 63,000 dalton protein might therefore be of viral origin. However, the fact that a protein with the same molecular weight and the same isoelectric point was also induced in fresh mouse embryo fibroblasts indicated that the protein is rather of cellular origin. Our results do not allow to conclude that the 63,000 dalton protein is fully induced by interferon; it may as well be a constitutive protein, whose synthesis is enhanced by interferon treatment. Thus, in our experiments the protein may have been present in the control cells in a non-labelled form. Further investigation will also be needed to determine the cell fraction with which the protein is associated and assign a specific function to it. It may be speculated that it corresponds to the antiviral protein whose existence was postulated by Taylor (1,2) and that it possesses one of the many biological activities described in lysates of interferon-treated cells (3-15). Two enzyme systems in these lysates, the $(2'\text{-}5')A_n$ synthetase and the protein kinase activity, are only activated in the presence of ds-RNA and both enzymes have been shown to have affinity for poly(I).poly(C)-Sepharose (13). In preliminary experiments we found that the radio-labelled 63,000 dalton protein also binds to poly(I).poly(C)-Sepharose. However, several other proteins from the lysate also adsorbed to a certain extent.

Note : After the submission of this paper other authors have reported similar autoradiographical vizualisation of interferon-induced proteins in human (22) and chick cells (23).

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