Characterization of Interferon Messenger RNA from Human Lymphoblastoid Cells

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SUMMARY

After treatment with Sendai virus, Namalwa cells form large amounts of interferon. RNA extracted from treated whole cells or from their polysomes was injected into *Xenopus laevis* oocytes and the interferon formed was titrated. The results show that the amount of interferon mRNA was maximal by 9 h after treatment of the cells with Sendai virus and then declined. Sucrose gradient centrifugation of the mRNA gave substantial purification and showed that its size was 12 S.

Interferons are proteins or glycoproteins that have an antiviral activity which is expressed with a characteristic species specificity. Human interferons can be divided into at least two types which may be distinguished by their antigenic and species specificity as well as by their mol. wt. distributions. One type, termed F, is produced by diploid fibroblast cells, while another antigenic type is the major component of the interferon formed by leukocyte and lymphoblastoid cells (see Burke, 1977). The two types of interferon are coded for by two distinct mRNAs, since translation of the mRNAs in *Xenopus* oocytes yielded interferons that were characteristic of the cells from which the mRNA was derived (Cavalieri et al. 1977a). There has been considerable interest in the mechanism of interferon induction because it is a model system for the control of gene expression in animal cells (see Burke & Morser, 1978). So far this approach has focused on the control of interferon production in human fibroblasts treated with double-stranded RNA. In this paper we report some of the properties of interferon mRNA formed in human lymphoblastoid cells induced with Sendai virus.

Human lymphoblastoid cells of the Namalwa line were grown in suspension culture and induced with Sendai virus as will be described elsewhere (P. N. Baker, J. Morser & D. C. Burke, manuscript in preparation). Total RNA was extracted from cells at various times after induction: cells were pelleted, washed twice with cold phosphate buffered saline and lysed in 4 M-guanidinium isothiocyanate, 1 M-2-mercaptoethanol in 20 mM-sodium acetate, pH 5·0. The lysate was layered over a 5·7 M-caesium chloride cushion and centrifuged overnight at 137000 g. The RNA formed a pellet, which was resuspended in 5 mM-tris/HCl, pH 7·5, and re-precipitated three times with three vol. of ethanol. Polysomes were prepared by the method of Schreier & Staehelin (1973) and RNA was extracted from them by the technique of Parish & Kirby (1966). RNA was analysed by sedimentation on sucrose gradients at 4°C in 10 mM-tris/HCl, pH 7·5, 100 mM-LiCl, 1 mM-EDTA and 0·1 % sodium dodecyl sulphate. Two gradients were used sequentially: first, a 7 to 30 %, w/v, exponential gradient centrifuged in an 8 x 25 ml angle rotor for 3 h at 230000 g; second, a 15 to 30 %, w/w, linear gradient centrifuged for 16 h at 190000 g in a 6 x 14 ml swing-out rotor. The gradients were harvested by upward displacement and u.v. absorbance was monitored; RNA was re-precipitated from appropriate fractions three times with three vol. of ethanol. RNA (5 mg/ml) was injected into *Xenopus*...
Fig. 1. Interferon production by Namalwa cells after induction with Sendai virus. (a) The interferon yield in the medium (●—●), in the cells (10⁶ cells/ml; □—□), and on the polysomes (10 A₂₆₀/ml; ○—○) at the times shown; the interferon yields 24 h after induction when either 1 µg/ml actinomycin D (▲—▲) or 50 µg/ml cycloheximide (■—■) was added at the times shown are also given. (b) The interferon yield from *Xenopus laevis* oocytes after injection of either total cellular RNA (●—●) or RNA from polysomes (○—○), extracted at the indicated time post-induction. *Xenopus laevis* oocytes by the published technique (Moar *et al.* 1971). The oocytes were incubated for 20 h at 23 °C before they were frozen at −70 °C, thawed and homogenized in 1 ml of Eagle’s minimal essential medium (Glasgow modification) plus 10 % calf serum and centrifuged to remove debris. Interferon assays were carried out as previously described (Atherton & Burke, 1975) in human foreskin fibroblast (HFF) cells or in human cells trisomic for chromosome 21 (GM 258) obtained from the Human Genetic Mutant Cell Repository, Camden, New Jersey, U.S.A. All assays included an internal standard, which had in turn been calibrated against the human leukocyte reference research standard (69/19) containing 5000 standard research units, which gave an endpoint titre of 2500 in HFF and of 15000 in GM 258 cells. Assay results are expressed in reference research units.

After induction with Sendai virus, interferon could be detected in the incubation medium within 3 h, the yield rising to a maximum after 12 h (Fig. 1a), whereas intracellular titres rose to a peak and then declined. The interferon associated with the polysomes also rose to a maximum by 9 to 10 h after induction before declining. This interferon associated with the polysomes was not totally adventitious, since, if cells that had not been induced with Sendai virus were resuspended in medium containing 10⁻¹² units/ml of interferon and the polysomes then isolated, only 10⁻⁴⁵ units of interferon/10 A₂₆₀ units of polysomes could be detected compared with 10⁻⁴⁷ units/10 A₂₆₀ units at the 10 h peak.

Further information about the time course of the production of interferon and its mRNA was obtained by measuring the effect on the 24 h yield of interferon of adding either actinomycin D (1 µg/ml) or cycloheximide (50 µg/ml) at different times after induction. Since these inhibitors prevent the further transcription or translation of the interferon mRNA, respectively, the final yields were determined by the amount of mRNA that had been transcribed or the amount of interferon that had already been formed at that time. The results (Fig. 1a) showed that the time course of the cycloheximide effect was very similar to that of the time course of the interferon titre in the medium, so that interferon was released shortly after synthesis. The time course of the effect of actinomycin D showed that the synthesis of interferon mRNA was similar to but preceded that of interferon by about 4 h. The curve suggests that interferon mRNA synthesis was complete by 5 h after induction. Similar results were obtained by Zoon *et al.* (1978).

Further information was obtained by measuring the amount of interferon mRNA
directly by injecting the RNA into oocytes and titrating the interferon formed. The results (Fig. 1b) showed that the amount of interferon mRNA, whether measured by injection of total cellular RNA or RNA extracted from polysomes, was maximal at 9 to 10 h after induction and then declined. A similar rise and fall has been reported for interferon production induced by treatment with poly(rI).poly(rC) (Cavalieri et al. 1977b). The interferon mRNA was found to be sensitive to ribonuclease (0.1 μg/ml for 10 min at 30 °C in 0.15 M-NaCl, 0.015 M-sodium citrate) and contained poly(A) and the interferon produced was active in human and bovine cells but not mouse or chick cells (data not shown). No interferon was formed in *Xenopus* oocytes when RNA from uninduced cells was injected.

The interferon mRNA was further characterized and partially purified by sequential centrifugation on two sucrose gradients. When the peak from the first gradient (Fig. 2a) was re-run on a second gradient (Fig. 2b), a peak at the same S value (12S) was obtained. There was no u.v. absorbing material associated with the biological activity, showing that substantial purification had been achieved.

In conclusion, we have shown that the production of interferon by Namalwa cells treated with Sendai virus is associated with an increase in the amount of interferon mRNA, whether measured in total cellular RNA or in polysomal RNA. Measurement of interferon associated with polysomes or of the amount of interferon mRNA in oocytes showed that the concentration of interferon messenger increased until 9 to 10 h p.i. This result was different from that obtained using actinomycin D, which had no effect after 5 h. We suggest that the result obtained with actinomycin D may be complicated by a processing step required in the formation of active interferon mRNA, or by some secondary effect of the antibiotic. Interferon production ceases because there is no longer any interferon mRNA associated with the polysomes. This suggests that the control of interferon production is similar to that found in human fibroblasts stimulated with poly(rI).poly(rC). The interferon mRNA had approximately the expected size to code for a protein of mol. wt. about 20,000. Our results suggest a route for substantial purification of interferon mRNA with a view to genetic engineering.
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