ly collected neotenes is correlated with the lowest tail height. Tail height is readily increased by treatment with ovine PRL or reduced by treatment with such ergot alkaloids as ergocornine and ergocryptine. These drugs enhance the sensitivity of neotenes to thyroid hormones, but this treatment in itself will not induce metamorphosis in the absence of exogenous thyroid hormones.
13. M. E. Hadley, V. J. Hruby, Sr. A. Bower, *Gen. Comp. Endocrinol.* **26**, 24 (1975).
14. J. Meites, K. H. Lu, W. Wuttke, C. W. Welsch, H. Nagasawa, S. K. Quadri, *Recent Prog. Horm. Res.* **28**, 471 (1972); C. M. Morgan and M. E. Hadley, *Neuroendocrinology*, in press.
15. W. A. Gern, thesis, Univ. of Colorado (1976).
16. Neotenes or immature larvae on long photoperiods [16 hours light : 8 hours darkness (16L : 8D)] show a significantly greater thyroidal radioiodide uptake than animals on short photoperiods (12L : 12D or 8L : 16D), although this is generally below that observed for actively metamorphosing animals. These data suggest that environmental factors might be able to alter thyroid hormone levels sufficiently to overcome the PRL block in the hypothalamus.
17. We would like to thank Dr. Richard E. Jones for his thoughtful criticisms of the manuscript and Sandoz Pharmaceuticals for the gift of ergocornine.

26 February 1976; revised 14 June 1976

Discharge of Aminoacyl–Viral RNA by a Factor from Interferon-Treated Cells

Abstract. *Extracts of cells treated with purified interferon discharge the amino acid previously esterified to viral RNA but fail to affect aminoacylated transfer RNA. A similar activity is also present in crude interferon preparations.*

The RNA from several different viruses can react in place of transfer RNA (tRNA) in the esterification of specific amino acids by eukaryotic aminoacyl-tRNA synthetases (*1*). The biological role in viral replication of such aminoacylation is not yet known, but the suggestion has been made that it may be involved in the regulation of protein synthesis (*2*). Interferon, a protein produced by cells exposed to viruses and certain other materials, causes cells to become resistant to viral infection and also alters specific functions of the treated cells, including protein synthesis (*3*). Incubation of exogenous messenger RNA (mRNA) with extracts of interferon-treated cells results in the translation of less protein than is observed with extracts of untreated cells; this low level of translation can be increased by the addition of certain tRNA preparations (*4, 5*). We report here another activity shown by extracts of cells exposed to interferon: specific deacylation of aminoacylated viral RNA.

The RNA extracted from tobacco mosaic virus (TMV) was esterified with histidine by partially purified rabbit liver histidinyl-tRNA synthetase. The unpurified human, rabbit, and mouse interferons used had all been induced in cell culture by virus; the residual virus was inactivated by dialysis at *p*H 2, and the neutralized fluids were then stored at −70°C. Mock preparations of unstimulated cell cultures were similarly prepared from supernatant fluids that, like the interferon preparations, contained 2 percent fetal bovine serum in Eagle's minimal essential medium.

In order to show that a factor interfering with aminoacylation of viral RNA is produced by cells after exposure to interferon, mouse L_{929} cells grown in 60-mm petri plates were treated with Sepharose beads bearing covalently linked mouse interferon (30,000 beads per dish) (*6*); control cells were treated with unmodified Sepharose beads. After incubation at 37°C for 6 hours, the beads were removed and the cells were washed and homogenized; supernatant fluids from the homogenates were then added either to an acylating system (Fig. 1a) or to a solution of histidinyl–TMV-RNA (Fig. 1b). As seen in Fig. 1, the extracts of cells that had been treated with interferon diminished the esterification of the TMV-RNA and increased the breakdown of preformed histidinyl–TMV-RNA.

The deacylating factor may be related to the development of resistance to viruses. Cells treated with Cantell's highly purified human interferon (*7*) (PIF) (with a specific antiviral activity of 3.7×10^6 units per milligram of protein) became progressively more resistant to virus challenge; the activity in extract supernatants increased in parallel with antiviral resistance until about 7 hours after interferon treatment, when deacylating activity began to decline and a plateau in antiviral resistance was reached (Fig. 2). The dissociation of the curves after 7 hours may be explained by mechanisms for disposing of the factor, such as binding to insoluble components or secretion from the cell.

Crude interferon preparations also contain a deacylating activity. The activity is clearly not attributable to interferon itself: no deacylating activity could be detected in as much as 170,000 units of PIF, but an active factor was present in crude preparations of human, rabbit, and mouse interferons (even after they were diluted > 1 : 250). The deacylating activ-

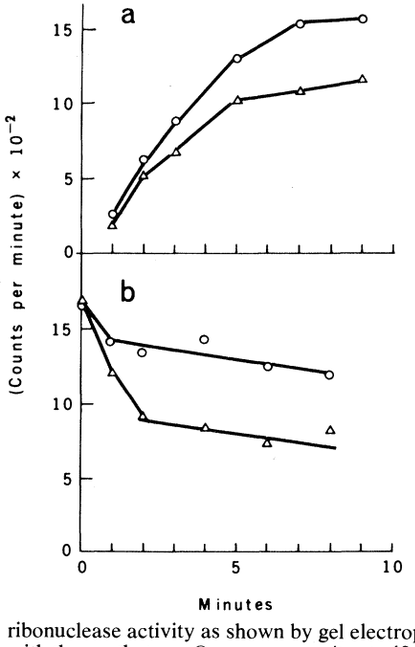

Fig. 1. The in vitro activity of L_{929} cell homogenates treated with Sepharose-bound mouse interferon (△) or with control, untreated Sepharose beads (○) on (a) the inhibition of histidine acylation to TMV-RNA and (b) the accelerated discharging of histidine TMV-RNA. The amount of interferon bound to the beads was about 3×10^4 units, as measured in L_{929} cells with GDVII virus as challenge (*12*). Cells were exposed to the beads for 6 hours at 37°C, then washed, homogenized in 1 ml of 0.02*M* tris-HCl buffer (*p*H 7.5), and centrifuged for 20 minutes at 2000*g*. Supernatant fluids were assayed. Rabbit liver histidinyl-tRNA synthetase was prepared from the 105,000*g* supernatant fluid of clarified liver homogenates. The fraction eluted from diethylaminoethyl (DEAE)-cellulose with 0.25*M* NH_4Cl served as a crude synthetase preparation from which the histidine synthetase was purified by column chromatography on DEAE-cellulose followed by affinity chromatography on a tRNA (*Escherichia coli*)–Sepharose column, and subsequently by TMV-RNA-Sepharose column chromatography, according to methods described by Remy *et al.* (*13*). Although not homogeneous, this preparation was devoid of ribonuclease activity as shown by gel electrophoresis of TMV-RNA before and after treatment with the synthetase. One enzyme unit esterifies 1 pmole of tRNA in 10 minutes. The TMV-RNA was prepared from a CsCl-banded virus preparation, which was then further concentrated, suspended in 1 percent sodium dodecyl sulfate, and extracted with an equal volume of phenol. The TMV-RNA was esterified with histidine in the following reaction mixture (0.1 ml): 0.02*M* tris-HCl (*p*H 7.5), 5 m*M* $MgCl_2$, 2 m*M* ATP, 10 μM [^{3}H]histidine, 4 to 10 pmole of TMV-RNA per 0.1 ml, 10 μl of extract from treated L_{929} cells, and 20 units of purified histidine synthetase. Aminoacylation was measured as trichloroacetic acid–precipitable tritium at the times indicated. Discharging was measured in a system containing 1 pmole of histidinyl–TMV-RNA in 0.02*M* tris (*p*H 7.5), 5 m*M* $MgCl_2$, 2 m*M* ATP, and 10 μl of cell extract. Samples were precipitated with trichloroacetic acid at the times indicated, filtered on glass fiber disks, washed, and counted in a scintillation counter.

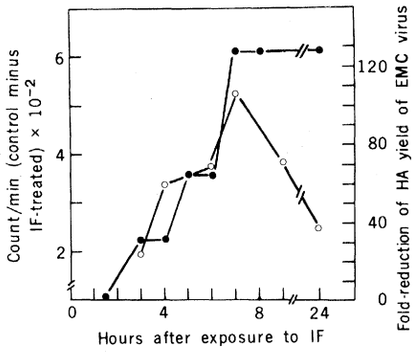

Fig. 2. Correlation of appearance of the deacylating factor with development of the antiviral state after interferon treatment. Rabbit kidney (RK-13) cells were treated with 10,000 units of PIF (highly purified human leukocyte interferon) at 36°C for the indicated periods of time. At the indicated times cells were either (i) washed three times and infected with a high multiplicity of encephalomyocarditis (*EMC*) virus for subsequent measurement of single-cycle viral hemagglutinin (*HA*) yield (●) or (ii) washed and extracted for determining deacylating activity (○) as in Fig. 1. *IF*, interferon.

ity was destroyed in a preparation of rabbit interferon by heating at 50°C, whereas the activity that stimulates antiviral resistances (interferon) survived heating to 70°C. Deacylating activity was also lost from a crude human interferon preparation that retained its ability to induce antiviral resistance after storage at −20°C and multiple freeze-thawings. Thus crude, dialyzed, *p*H 2–treated interferon preparations are mixtures of materials released by cells after exposure to virus, including the deacylating factor produced by some cells in response to the interferon produced by other cells.

Assays of deacylation in interferon preparations necessarily included comparisons with preparations of mock interferon as controls because sufficient quantities of mock preparation also can decrease the acylation of TMV-RNA. However, when appropriate dilutions are used, the preparations of mock interferon have little effect on the rate of acylation, and the difference between mock and interferon preparations is proportional to the amount of the crude interferon preparation added (Fig. 3). Discharging of histidinyl–TMV-RNA is dependent on the presence of adenosine triphosphate (ATP), with maximum stimulation at about 2 m*M*.

An experiment was designed to determine whether the deacylating factor itself discriminates between tRNA and viral RNA or whether a material in the mixtures of crude extracts and TMV-RNA affects all aminoacylated RNA. The TMV-RNA and tRNA were mixed and added to incubation mixtures containing histidinyl-tRNA synthetase and either the crude rabbit interferon or the mock preparation. The results, shown in Fig. 4, indicate that the deacylating factor decreases selectively the amount of aminoacyl–viral RNA and does not cause a change in the amount of cognate aminoacyl-tRNA. An indirect effect (for example, inhibiting the synthetase or activating a nuclease) would be expected to affect aminoacylation of both species of RNA.

A plant antiviral preparation (*8*) affects aminoacyl–TMV-RNA in the same manner as interferon-treated cell extracts and crude interferon preparations (data not shown). Further, our data show that encephalomyocarditis virus RNA, esterified with serine, as reported by Stebbing and Lindley (*9*), is also deacylated by the interferon preparations that deacylate histidinyl–TMV-RNA. Thus, it appears that a deacylating factor is stimulated as a response of various cells to appropriate antiviral factors and that this factor recognizes the aminoacylated RNA of quite different viruses. Under our experimental conditions histidinyl-tRNA and seryl-tRNA are resistant to the factor. Moreover, several other amino acids can be esterified to their cognate tRNA's just as well in the presence of the factor as in its absence. An effect of interferon treatment on Ehrlich ascites cells has recently been reported by Sen *et al.* (*10*) to decrease the aminoacylation of some of

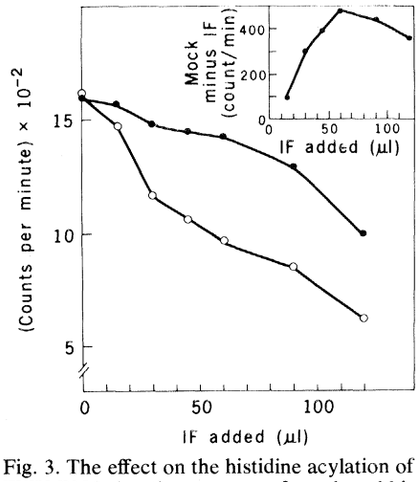

Fig. 3. The effect on the histidine acylation of TMV-RNA by the amount of crude rabbit interferon preparation added to incubation mixtures described in Fig. 1. The TMV-RNA was esterified with histidine in the presence of the mock control (●) or the crude rabbit interferon diluted 1:600 (○). The inset shows the inhibition of TMV esterification by the crude interferon preparation as the calculated difference from the control in the large figure plotted against the amount of crude material added. The rabbit interferon had an antiviral potency of 1.7×10^5 units per milliliter measured in RK-13 cells, with encephalomyocarditis virus as challenge. *IF*, interferon.

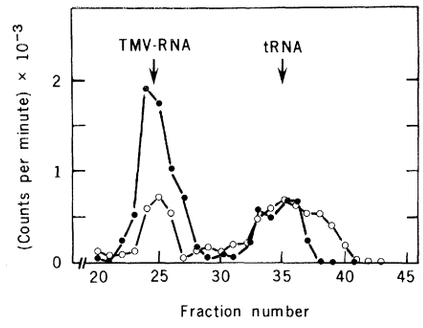

Fig. 4. Selectivity of inhibition of aminoacylation of TMV-RNA or rabbit tRNA by crude rabbit interferon (used in Fig. 3) or the mock control. An equimolar mixture of TMV-RNA and tRNA was prepared, and the RNA was esterified with [^{3}H]histidine as in Fig. 1 in the presence of a 1 : 2000 dilution of crude rabbit interferon (○) or its mock preparation (●), followed by isolation of the aminoacylated RNA's. Marker tRNA and TMV-RNA were added to permit identification of fractions by ultraviolet light absorption, and the mixture was separated on a column of Sephadex G-100. The source of the rabbit tRNA was a phenol extract of the 105,000*g* supernatant fluid of clarified liver homogenates. The RNA was precipitated from the aqueous phase with ethanol, resuspended in 2*M* LiCl, clarified by centrifugation, and reprecipitated with ethanol.

the endogenous tRNA's; it is not yet possible to relate this phenomenon to the discharging described here. Similarly, we cannot tell whether deacylation is involved in the decrease of translation caused by interferon (*5, 11*).

The phenomenon described here may be useful as a rapid, simple in vitro assay of an interferon-induced factor, thereby permitting the identification of material (or materials) produced in response to interferon.

ILAN SELA, SIDNEY E. GROSSBERG
J. JAMES SEDMAK, ALAN H. MEHLER
Departments of Biochemistry and *Microbiology, Medical College of Wisconsin, Milwaukee 53233*

References and Notes

1. P. Yot, M. Pinck, A. Haenni, H. M. Duranton, F. Chapeville, *Proc. Natl. Acad. Sci. U.S.A.* **67**, 1345 (1970); T. C. Hall, D. S. Shih, P. Kaesberg, *Biochem. J.* **129**, 969 (1972); B. Oberg and L. Philipson, *Biochem. Biophys. Res. Commun.* **48**, 927 (1972); I. Sela, *Virology* **49**, 90 (1972).
2. D. S. Shih, P. Kaesberg, T. C. Hall, *Nature (London)* **249**, 353 (1974); R. Salomon and U. Z. Littauer, *ibid.*, p. 32.
3. S. E. Grossberg, *N. Engl. J. Med.* **287**, 13, 79, and 122 (1972); M. Ho and J. A. Armstrong, *Annu. Rev. Microbiol.* **29**, 131 (1975).
4. E. Falcoff, R. Falcoff, B. Lebleu, M. Revel, *Nature (London) New Biol.* **240**, 145 (1972); S. L. Gupta, M. L. Sopori, P. Lengyel, *Biochem. Biophys. Res. Commun.* **54**, 777 (1973); *ibid.* **57**, 763 (1974).
5. M. Revel, J. Content, A. Zilberstein, B. Lebleu, E. Falcoff, R. Falcoff, in *Perspectives in Virology*, vol. 9, *Antiviral Mechanisms*, M. Pollard, Ed. (Academic Press, New York, 1975), pp. 233–247.
6. H. Ankel, C. Chany, B. Galliot, M. J. Chevalier, M. Robert, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2360 (1973).
7. K. Cantell, S. Hirvonen, K. E. Mogensen, L. Pyhälä, in *The Production and Use of Interferon*

for the Treatment and Prevention of Human Virus Infections, C. Waymouth, Ed. (*In Vitro* Monograph No. 3, Tissue Culture Association, Rockville, Md., 1974), pp. 35–38.
8. I. Sela, I. Harpaz, Y. Birk, *Virology* **28**, 71 (1966); I. Sela, unpublished observations.
9. N. Stebbing and A. Lindley, personal communication.
10. G. C. Sen, S. L. Gupta, G. E. Brown, B. Le bleu, M. A. Rebello, P. Lengyel, *J. Virol.* **17**, 191 (1976).
11. E. Samuel and W. Joklik, *Virology* **58**, 476 (1974).
12. H. Oie, C. Buckler, C. P. Uhlendorf, D. A. Hill, S. Baron, *Proc. Soc. Exp. Biol. Med.* **140**, 1178 (1972).
13. P. Remy, C. Birmele, J. P. Ebel, *FEBS Lett.* **27**, 134 (1972).
14. We thank Dr. Kari Cantell for supplying crude and purified human interferon preparations, Dr. Pat Jameson for providing rabbit interferon, Dr. Helmut Ankel for providing Sepharose-bound interferon, and Ms. Françoise Besançon for her assistance and for providing mouse interferon. I.S. was on sabbatical leave from the Virus Laboratory, Hebrew University Faculty of Agriculture, Rehovot, Israel. This work was supported by NIH (N01 AI 02125, N01 AI 42514, and 5R01 GM 13037) and NSF (BG-27440A1) awards.

11 May 1976; revised 12 July 1976

Ribosomal Genes of *Xenopus laevis*: Evidence of Nucleosomes in Transcriptionally Active Chromatin

Abstract. *Most of the reiterated ribosomal genes in the somatic cells of larvae of a mutant of* Xenopus laevis *appear to be protected from short-term nuclease digestion by being packaged in the form of chromatin subunits or nucleosomes. Since these mutant animals probably require all of their ribosomal genes to be active in order to maintain viability, at least some of the transcriptionally active gene sequences are probably associated with chromatin subunits. Thus, association of DNA with nucleosomes may not necessarily preclude template activity, although such association is probably of a dynamic rather than a static nature.*

Evidence from a wide variety of sources (*1*) indicates that most of the chromatin of higher eukaryotes is packaged into repeating arrays of globular subunits consisting of double-stranded DNA associated with histones separated by stretches of DNA not packaged in this globular form. A model for these fundamental chromatin particles, also called nucleosomes (*2*) or nu-bodies (*3*), has been proposed by Kornberg (*4*). In this model about 200 base pairs of DNA are arranged on the outside of a globular octamer of histones with the composition $(H4)_2(H3)_2(H2A)_2(H2B)_2$, formerly known as $(F2A1)_2(F3)_2(F2A2)_2(F2B)_2$. A similar nucleosome model has been proposed by Van Holde *et al.* (*5*). Most recent evidence has supported such an arrangement of histones and DNA (*6*) in both native (*7*) and reconstituted (*2, 8*) chromatin.

Indirect evidence suggests that transcriptionally active (as well as inactive) chromatin may be packaged, at least partially, into the nucleosome configuration (*9–19*). In all of the studies cited, however, the starting population of cells from which the chromatin subunits were released by nuclease digestion appeared to be heterogeneous with respect to genomic transcriptional activity. Thus, the nucleosomes derived from such mixed populations could well have been released from transcriptionally inactive gene sequences present within the starting population.

Here I present evidence from nucleic acid hybridization studies that a major fraction of the reiterated ribosomal genes in the somatic cells of a mutant of the amphibian *Xenopus laevis* is protected from short-term micrococcal nuclease digestion by being packaged into nucleosomes. To maintain viability, larvae heterozygous for this mutation (the anucleolate mutation) probably require all of their ribosomal cistrons to be synthetically active (*20, 21*), suggesting that transcriptionally active ribosomal DNA may be partially associated with histones in much the same manner as transcriptionally inactive chromatin (*9*). However, this association may be rather labile, as will be shown.

There are about 450 to 500 adjacent sets of ribosomal genes per haploid genome in wild-type *Xenopus laevis* (*22, 23*). This corresponds to about 0.2 percent of the nuclear DNA of diploid wild-type somatic cells (*24*). Each of these tandemly repeated ribosomal cistrons includes DNA which codes for a 40*S* RNA transcript that is a precursor to 28*S* and 18*S* ribosomal RNA (rRNA) as well as DNA for a nontranscribed "spacer" region of high deoxyguanylic and deoxycytidylic acid (G + C) content (*22, 24*). In diploid wild-type animals the 1000 or so cistrons are distributed equally between two nucleolar organizer regions located on homologous chromosomes (*25*). Such animals usually have two nucleoli per nucleus during early larval stages of development and are hence designated as +/+ nu (*26*).

In a certain strain of these animals there is a stable mutation, the anucleolate mutation, which involves the total deletion of the repeated ribosomal genes from the nucleolar organizer region of the chromosome (*22, 27*). Animals heterozygous for this mutation (0/+ nu individuals) have only one nucleolus per nucleus but are viable even though they have only half of the number of ribosomal genes (about 500) of the wild type. On the other hand, animals homozygous for the anucleolate mutation (0/0 nu individuals) have no detectable ribosomal DNA in their genomes (*24*), never synthesize any rRNA (*28*), and die at an early larval stage of development [stage 41 or 42 (*26*)].

In all embryos of *Xenopus* which have ribosomal cistrons in their genomes, rRNA synthesis commences only after the early gastrula stage of development has been reached [stage 10 or 11 (*29*), some 10 to 12 hours after fertilization (*30*)]. From this time onward the rate of synthesis appears to increase continuously until about the heartbeat stage of development when it begins to slow down and reaches a maximum plateau sometime around stages 42 to 45 (the feeding tadpole stage) of development (*26, 30*). By this stage of development the heterozygous +/0 nu animals are synthesizing rRNA at the same absolute rate as are +/+ nu wild-type animals (*21, 28*). This compensation in rates of synthesis by the heterozygotes can be explained either by assuming that wild-type animals have only about half of their cistrons transcriptionally active or by postulating that the +/0 nu individuals are synthesizing rRNA at twice the wild-type rate on all of their cistrons (*21*). In either case, all of the cistrons of the +/0 nu animals are probably transcriptionally active, because a reduction of the number of ribosomal cistrons below the heterozygous level (as shown in a series of partial deletion mutations) results in larval death (*20, 21*).

The nature of the structure within transcriptionally active chromatin was investigated by means of two types of experiments: (i) Radioactive rRNA was hybridized to purified monomer nucleosome (monosome) DNA fragments (about 200 base pairs in length) derived from micrococcal nuclease-treated nuclei obtained from heterozygous +/0 nu tadpoles (stage 42 or 46) engaged in maximal rates of rRNA synthesis. (ii) Similar saturation hybridization experiments were conducted with monosome DNA fragments from early embryonic stages just before (blastula, stage 8 or 9) and just after (neurula, stages 16 to 18) the onset of rRNA synthesis. In the latter experiments, all of the animals resulting from +/0 nu × +/0 nu matings were used because of the difficulty in identifying the