alone might be visible even in later stages of the viral replication. Experiments showed an effect of cAMP at all times, mostly pronounced during maximal speed of replication around the 7th to 9th h of infection.

Our findings extend the few observations made by others. Replication of adenovirus, type 2, is inhibited by dbcAMP; the cell line itself has an effect[15]. An increased antiviral activity of Interferon in CEF infected with SFV was mentioned[16]. However, the following findings are new: a) cAMP has an antiviral activity of its own in our system; b) cAMP acts also in the late phases of replication of SFV; c) cAMP has probably no inducing activity in respect to antiviral resistance development. In the figures and tables of Weber and Stewart[8], showing cAMP potentiation of Interferon, marginal effects of cAMP alone - reducing vesicular stomatitis plaque numbers in L-cells to 80% at maximum -, can be seen.

With respect to the cooperative effects of Interferon and cAMP, however, there is a clear difference between our results and theirs[8]: they show a synergistic effect on plaque reduction of exogenously added Interferon and cAMP, whereas we find an at most additive effect, disappearing at higher concentrations of Interferon. Virus-host systems might play a role.

Interestingly enough, our cAMP effects are in contrast to those in uninfected CEF[14], where the biosynthesis of all RNA types is stimulated after an induction time of about 3 h. Our results are compatible with the view that Interferon acts at the cell membrane at a site where the adenylate-cyclase is influenced; however, they do not prove a causal relation between Interferon and cAMP. An influence of cAMP on Interferon synthesis after the challenge with the test virus is a remote possibility[17-19].

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Altered or increased transfer-RNA methylation in the course of Interferon action on cells in culture?[1]

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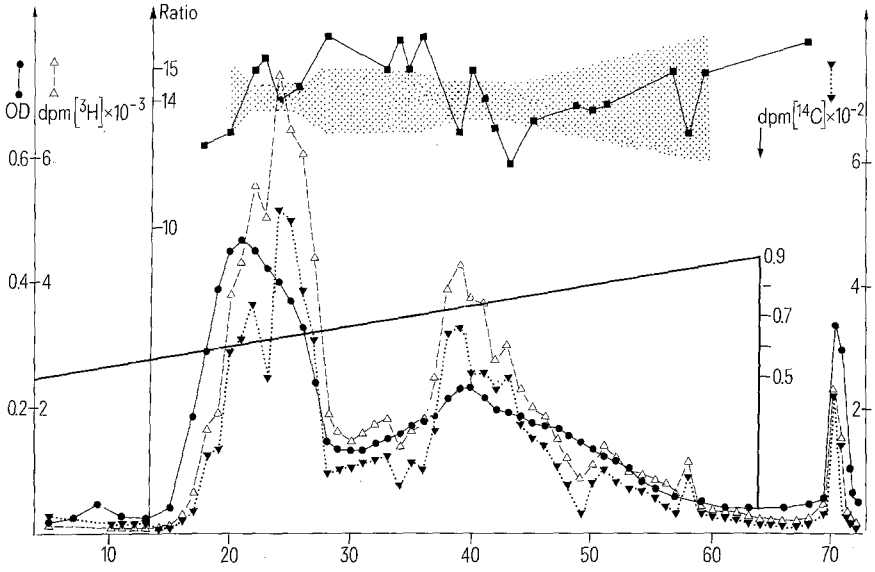
Summary. The induction of the antiviral state by Interferon might reflect the decrease of the rate of biosynthesis, the degradation or the alteration of one or several tRNAs. This could result in rate-limiting concentrations for codons common in viral RNA but rare in host mRNA. Altered methylation of tRNA could be the basis of such a phenomenon. However, we could not find an altered extent of methylation of total tRNA or an altered pattern of methylation, if mixed tRNAs were chromatographed on MAK- or BD-cellulose columns, despite a large range of conditions of pretreatment of chick embryo fibroblast cultures with interferon.

The regulation of protein biosynthesis with rate-limiting function or concentration of certain tRNA species has been described[4,5]. It is therefore tempting to speculate that cells could be protected against viral infections by lowering function or concentration of tRNAs serving codons, which are common in viral RNA in comparison to host RNA. Interferon could be a substance which induces such a type of regulation. Some papers indicate a connection between Interferon and tRNA[6-12]. A possible explanation for these observations is offered by the finding that Interferon stimulates tRNA-methylase enzymes[13] assayed in vitro. There is evidence that the distribution of methylgroups in tRNA governs the efficiency with which these molecules accept aminoacids and act as donors[14,15]. The main effects were found if non-homologous tRNAs were used as acceptors in this assay in vitro[13]. Therefore we wanted to know more about the methylation of tRNA in vivo (with homologous tRNAs) after treatment with Interferon.

Materials and methods. Cell cultures, media and infection with Semliki Forest Virus (SFV), Zurich strain, were as described[16,17]. Interferon preparations were those used earlier[18]. Antiviral activities were assayed as described[16,17].

Extent of tRNA-methylation: In the evening before the start, chick embryo fibroblast (CEF) cultures received Eagle's medium with $^1/_{10}$ of the normal aminoacid concentration ($^1/_{10}$ Eagle) to deplete the 1C-fragment pool, 10^{-4} M



Chromatographic pattern of tRNA on BD-cellulose columns. 1 set of CEF-cultures was pretreated with mock-Interferon, 1 set with Interferon (25 units/ml) over night. 1 half of each set was then labelled with [^{3}H]-uridine, the other half with [^{14}C]-uridine. tRNA was extracted, mixed in an activity ratio of 1 dpm [^{14}C]: 14 dpm [^{3}H] and brought onto a BD-cellulose column. The arrow indicates the final elution with ethanol. ●——●, absorbancy at 260 nm of the *Coli*-tRNA marker; △- -△, [^{3}H] dpm of tRNA from cultures with mock-Interferon; ▼....▼, [^{14}C] dpm of tRNA from cultures with Interferon. - The NaCl-concentration of the gradient is indicated with the right inner scale. Left inner scale (■——■), [^{3}H] dpm: [^{14}C] dpm ratio. The hatched area set into the ratio profile indicates the deviation of the ratio from the ideal horizontal at 14 based on counting statistics (± 1 SD for each label).

adenosine to inhibit incorporation of 1C-fragments into purine ring positions[20] and 3 μCi/dish of [^{3}H]-uridine to get a reference in tRNA. In some experiments Interferon was added in concentrations of 0.5–4 units/ml. In other experiments 0.5–4 units/ml of Interferon were given for 1–8 h before the start to find possible transitory influences. Experiments had shown that these treatments did not reduce the extent of incorporation of [^{3}H]-uridine into newly formed (t)RNA or the protection induced by Interferon.

At the beginning of the experiments, cultures were infected or mock-infected at 37 °C for 1 h (m.o.i. = 1–10) with SFV so that the following combinations of treatments resulted: mock Interferon plus mock infection; mock Interferon plus infection; Interferon plus mock infection; Interferon plus infection. Thereafter the medium was replaced with $^1/_{10}$ Eagle containing 10^{-4} M adenosine and 1–3 μCi [^{14}C-methyl]-L-methionine[16] to label methylgroups of tRNA (for 3 h at different times after infection or for different lengths of time). Pilot experiments had shown that total methyl-incorporation into tRNA increased linearly over more than 9 h after infection in the label-range stated. Latest harvest was 9 h after infection. Transfer-RNA was extracted with SDS and phenol, precipitated with ethanol and solubilized several times with 4 M LiCl, pH 7. Aminoacids were stripped off by incubation at pH 9 and 37 °C for 1 h in 0.2 M Tris HCl; then tRNA was reprecipitated with ethanol. It was further purified on 5–20% sucrose gradients in a SW 50.1 rotor (L2-50B) at 15 °C and 39,000 rpm for at least 3.5 h; the medium was 0.01 M sodium acetate, pH 5.1, 0.05 M NaCl, 1 mM EDTA, 50 mg/l PVS and 1% SDS. Total and specific radioactivities were compared. There are no viral fractions in the sucrose gradient region of tRNA.

Chromatographic patterns of tRNA: Cell cultures pretreated as above (but up to 25 units of Interferon/ml overnight) were labeled with [^{3}H]-uridine (4 μCi/dish) or [^{14}C]-uridine (0.1 μCi/dish) or [^{14}C-methyl]-L-methionine (2 μCi/dish) for 10 h. tRNA was extracted, stripped, mixed and all cross-combinations were chromatographed on methylated-albumin-Kieselgur (MAK)-[21] and BD-cellulose[22] columns. Separation of tRNAs on BD-cellulose seems to depend upon preferential interaction of the single-stranded regions with the aromatic rings bound to the cellulose. BD-cellulose is particularly apt to detect differences in methylation[23], though other modifications of tRNA could influence the elution also. The assumption is that differences in methylation of certain tRNAs should result in differences of profiles, even if individual tRNAs cannot be recognized in this way, because the aminoacid moiety is not labelled. In the case of BD-cellulose, the starting buffer was 0.05 M Na acetate, pH 5, 0.01 M $MgCl_2$, 0.5 M NaCl. Shallow gradients were made to 0.9 M NaCl and fractions of 2 ml were collected at room temperature. A final elution step followed with 1 M NaCl and 10% (v/v) ethanol. Samples contained 2 mg of tRNA from *E. coli* as absorbance marker. Eluted RNA was precipitated together with a salmon sperm DNA carrier with trichloroacetic acid and collected on Whatman GF/B glas fibre filters. Radioactivity was determined in a Packard spectrometer Tri-Carb 3380 with absolute activity analyser.

Results and discussion. Influence of Interferon on extent of tRNA-methylation in the course of tRNA biosynthesis: No significant differences could be found. This was true for specific activities (dpm from [^{3}H]-uridine or dpm from [^{14}C-methyl]-L-methionine per OD-unit of tRNA) or for total radioactivities incorporated. The conclusion is that neither time of presence nor concentration of Interferon influences the overall extent of methylation of tRNA.

Influence of Interferon on chromatographic patterns of tRNA: Though the overall extent of methylation remained unchanged, there is no proof that the extent of methylation of individual tRNAs corresponds to that of the control. A rigorous proof would only be possible with isolation of all individual isoaccepting species and oligonucleotide mapping[25]. However, at first, it seems sufficient to analyze total tRNAs in cross-combinations with chromatographic methods. No clear-cut differences in profiles could be detected. An example is given in the figure. This is in line with the finding[24] that profiles of leucyl-tRNA (labelled in the aminoacid moiety) from untreated and from Interferon-treated L cells on reversed-phase chromatography

columns No. 5 are identical. On the other hand, in cell-free systems, results are compatible with a model according to which some tRNA species, e.g. leucyl-tRNA, become limiting (extracts from Interferon-treated cells)[6,7]. Leucyl-tRNAs have been shown to be unstable in extracts prepared from Ehrlich ascites cells treated with Interferon[11]. An explanation for these conflicting results may be that the impaired function of certain tRNAs has no structural correlate which can be detected with our chromatographic means.

Despite the fact that the deviations of the isotope ratio in the figure are somewhat larger than allowed by the counting statistics, we think - based on the similarity of all profiles - that Interferon has probably no significant effect on tRNA methylation in our system. Kroath et al.[25] came to the same conclusion by analyzing the pattern of methylated nucleotides of tRNA of CEF. Even if methylase activities or concentrations are stimulated as reported[13], the normal levels may be sufficient fully to methylate homologous tRNAs. The concept of rate-limiting tRNA-function or concentration provoked by Interferon need not be wrong, but it would not be based on changes in methylation extent or pattern.

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Polyamines as activators of AMP nucleosidase from *Azotobacter vinelandii*

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Summary. Polyamines at physiological concentrations activate AMP nucleosidase from *Azotobacter vinelandii*. Biological significance of the activation is discussed in relation to the control of adenylate energy charge and the purine nucleotide synthesis in prokaryotes.

A vast amount of literature has accumulated on the role of polyamines related to increased cellular proliferation in animal and bacterial cells[1,2]. Polyamine accumulation is accompanied by an increase in the rate of RNA-synthesis as well as protein synthesis[2-5]. Furthermore, polyamines were demonstrated to be involved in the control of the activities of several enzymes[6-10]. Recently, we reported that polyamines at physiological concentrations activate the rat liver AMP deaminase (EC 3.5.4.6)[11], which may be important to stabilize the adenylate energy charge[12] and in the conversion of adenine nucleotides to inosine or guanine nucleotides[13]. Polyamines were suggested to participate in the stabilization of energy charge and the synthesis of purine nucleotides during cell proliferation of liver[11]. On the other hand, AMP nucleosidase (EC 3.2.2.4) is responsible for the regulation of the energy charge[14] and conversion of AMP to IMP in *Azotobacter vinelandii*[15,16] and probably also in other prokaryotic cells[12,14]. It is, thus, reasonable to assume that polyamines can activate the *Azotobacter* AMP nucleosidase, and this assumption was verified in the present paper. The physiological significance of the results is discussed in relation to the control of the energy charge and the purine nucleotide synthesis in bacterial cell growth.

The effect of polyamines and diamines, which differ from each other in the number of carbon atoms separating the 2 amino groups, on the AMP nucleosidase activity was examined. All the polyamines including diamines acted as powerful activators of the enzyme (figure). The activating effect of polyamines was suggested to depend upon the spatial separation of the primary amino groups: diamines separated by 5 or more carbon atoms as well as spermine and spermidine were the most effective activators of the enzyme. Polyamines are known to replace Mg^{2+} entirely or