Stimulation of Transcription in HEp-2 Cells by UV-Inactivated Poliovirus Type 1

The influence of infection with poliovirus type 1 on the quantitative binding of tritiated actinomycin-D (^{3}H-act.-D) by cellular chromatin of HEp-2 cells has been studied following a suggestion that the binding of the antibiotic to deoxyribonucleoprotein (DNP) may be used as a parameter of DNA template activity[1–4]. These data were correlated with results of experiments on the influence of poliovirus infection on the synthesis of 45 S percursor RNA[5,6] under similar experimental conditions.

Material. HEp-2 cells were cultured in spinner flasks (Bellco) in Eagle's minimum essential medium (MEM) (Joklik's modification, Gibco) containing 5 or 10% calf serum and penicillin (100 U/ml) and streptomycin (100 µg/ml). Poliovirus type 1 strain Mahoney was used for infection; UV-inactivation was adjusted to reduce the infectivity by 3 to 4 $\log_{10}$. ^{14}C-thymidine (TdR; Amersham, the specific activity was 59–62 mCi/mM) was diluted to 0.25 to 0.9 nCi. ^{3}H-act.-D (Amersham, the specific activity was 9.1 Ci/mM) was diluted to 54 nCi. Recovery from 10^6 cells (= 50 µg of DNA as determined by the diphenylamine-method) was approximately 3 nCi ($4\times10^{-13}M$). ^{3}H-uridine (UR; Amersham, the specific activity was 6.3 Ci/mM) was diluted to 40 nCi. Chase concentrations were 15 to 125 nM (TdR), 400 ng/ml (act.-D, Serva), and $10^{-8}M$ of each of UR, TdR and cytidine used after the ^{3}H-UR pulse.

Method. For experiments, HEp-2 cells were cultured in 'conditioned' medium consisting of 2 parts of freshly prepared growth medium and 1 part of supernatant medium previously used for cultivation of cells. Cells were infected by suspension in 5 to 7 ml of virus purified[7,8] in CsCl, which was diluted in growth medium without serum. Multiplicities of infection (MPI) are expressed as plaque-forming units (PFU)/cell and are given below for the individual experiments. Extraction of the acid-alcohol-resistant, DNase-sensitive fraction of HEp-2 cells labelled overnight with ^{14}C-TdR and used for the act.-D pulse (30 min)/chase (20 min) experiments has been described[4]. Cell nuclei were isolated after a UR pulse (5 min)/chase (10 min) as described[6]. The nuclear RNA was extracted with hot phenol (60 °C)[9], and centrifuged (15 h 4 °C 150.000 g_{max}) through a continuous 8 to 35% sucrose gradient. Fractions were collected from the bottoms of tubes. The typical OD (260 nm) profile was eliminated by exposing the extracted material to RNase (Worthington, heated 10 min at 100 °C before use). Radioactivity counts were corrected for quenching and counted in a Packard scintillation spectrometer.

Result. Cultures of HEp-2 cells were pulse/chased with UR 180 min after the beginning of poliovirus infection. The nuclear RNA was extracted, and the radioactivity of its subunits determined. When the cells were not infected or when they had been exposed to UV-inactivated viruses, the radioactivity was mainly associated with the heaviest RNA fraction, Figure 1. The synthesis of precursor RNA was significantly reduced after exposing the cells to live viruses.

The quantitative binding of act.-D to cellular chromatin was studied in cells exposed to live viruses (MPI 50 to 500), or to UV-inactivated viruses. Additional cultures were not infected at all. The ^{3}H-act.-D/^{14}C-TdR ratios were determined as described before[4], and the results were compared by calculating the 'inoculum ratios' of average ^{3}H/^{14}C ratios based on 3 chemical determinations, Figure 2. The inoculum ratios 'non-infected/infected' for various times after the beginning of the infection showed values close to 1.0. Cells exposed to UV-inactivated viruses showed a non-random increase of the binding of act.-D by 15 to 21% ($p < 0.05$ by t-test), as expressed by the comparisons 'inactive/active' and 'inactive/non-infected'.

Discussion. Ribosomal precursor RNA, a product of the transcription of discreet chromosomal locations, was inhibited by live polioviruses, while inactivated viruses had no effect. This agrees with results of experiments with polio-infected HeLa cells[10,11]. By contrast, there

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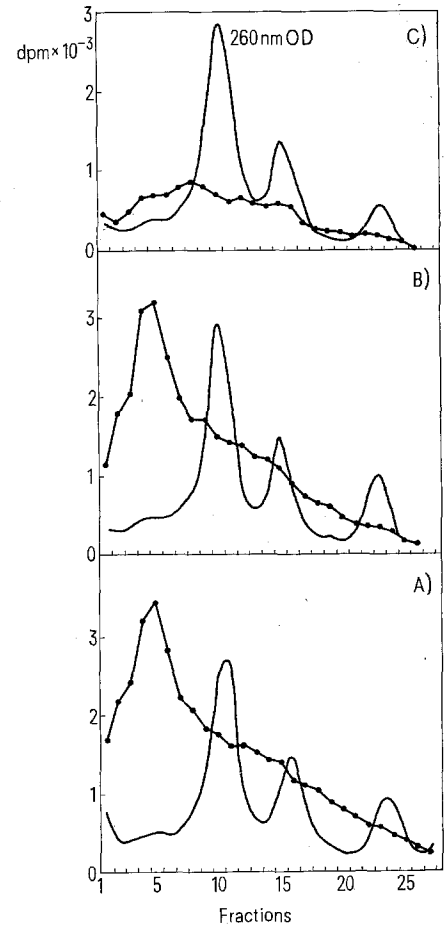

Fig. 1. Synthesis of nuclear precursor RNA by poliovirus-infected HEp-2 cells. The incorporation of ^{3}H-UR into precursor RNA was 4.1% of total pulse activity for cells exposed to UV-inactivated viruses (A), 3.6% for non-infected cells (B), and 0.95% for infected cells (C; MPI = 20).

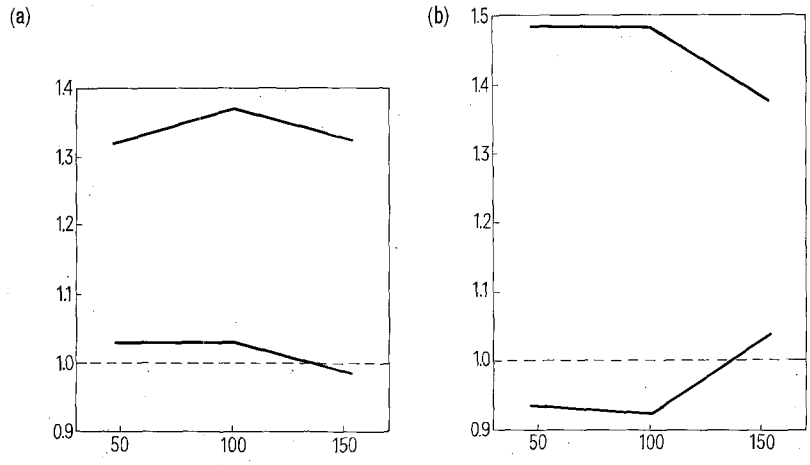

Fig. 2. 'Inoculum ratios' inactive/active (a) and inactive/non-infected (b) obtained from 12 experiments by comparing the ^{3}H-act.-D/^{14}C-TdR ratios for various times (min) after the beginning of the infection. The time symbols (abscissae) indicate the beginning of the pulse/chase; the lines the ranges of 2 standard deviations.

was no difference in the binding of act.-D by infected and non-infected cells, and the increase of binding after exposure to UV-inactivated viruses suggests a stimulation of DNA template activity in these cells. Act.-D specifically interferes with the production of RNA[12] by a mechanism involving intercalation of the chromophore of the antibiotic between G-C base pairs of the DNA double strand and chemical binding[2,3,13,14]. Assuming that act.-D plays the role of a non-specific model repressor[1-3], the quantitative analysis of its in vivo association with DNP may be considered a parameter for the earliest possible, albeit non-specific detection of changes of DNA template activity or, correspondingly, for the degree of complexing of DNA with chromosomal proteins[15], since transcription involves changes in the DNP complex[16,17].

The increase of binding of act.-D after exposure of cells to UV-inactivated viruses suggests that the viral coat protein was responsible for this stimulation, and that this process may be controlled by the intact viral genome.

Zusammenfassung. Nach Einwirkung UV-inaktivierter Polioviren wurde eine Zunahme der Bindung von Actinomycin-D, eines unspezifischen «Modellrepressors», durch HEp-2 Zellen um 15–21% beobachtet. Die Proteinhülle UV-inaktivierter Viren verursacht möglicherweise eine Stimulierung der Genaktivität der Wirtszelle, die normalerweise der Kontrolle des intakten Virusgenoms unterliegt.

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Lanthanum Inhibits Ca Inward Current but not Na-Ca Exchange in Cardiac Muscle

Two calcium transfer systems in the sarcolemma of mammalian cardiac muscle have been described: 1. a time- and voltage-dependent conductance system responsible for most of the inward charge transfer during the plateau phase of the cardiac action potential[1-4], and 2. a Na-Ca exchange system which is primarily responsible for extrusion of Ca from cardiac cells[5,6]. We report 2 series of experiments which provide additional evidence that these two Ca-transfer systems are separate, one being sensitive to external lanthanum ions while the other is not.

Methods. We carried out voltage clamp experiments in ventricular trabeculae (diameter 0.3–0.6 mm) isolated from pig and sheep hearts. The method, utilizing a sucrose gap for passing current through the preparation and intracellular microelectrodes for measuring and controlling the membrane potential, has been described previously[7]. The bathing solution had the following composition (mM/l): NaCl 137; KCl 5.4; $MgCl_2$ 1.05; $CaCl_2$ 1.8; glucose 5.0; *Tris*-HCl-buffer to pH 7.2 at 35 °C. $LaCl_3$ was added to give a final concentration of 0.4 mM/l.

For Ca efflux measurements guinea-pig auricles were loaded with ^{45}Ca in Tyrode's solution. The ^{45}Ca efflux from the resting auricles into nonradioactive solutions containing different Na- and Ca-concentrations was measured in the presence and absence of La (0.2-0.9 mM/l). The method has previously been described in detail[5].

Results and discussion. In the first series of experiments we studied the effect of La on the two components of inward current which flow during the cardiac action potential[4]. The first component is carried by Na ions (I_{Na}). In cardiac muscle, as in other excitable tissues, I_{Na} is rapidly

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