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BIKEN JOURNAL
Preliminary Report

Intracellular Distribution of ^{3}H-Actinomycin S in TG Cells Demonstrated by Autoradiography*

Actinomycin has been proved to inhibit DNA dependent cellular RNA synthesis (Hurwits *et al.*, 1962; Reich *et al.*, 1963, Levinthal *et al.*, 1963) presumably by interacting with the DNA molecule (Kawamata and Imanishi, 1960, Rauen *et al.*, 1960, Kirk, 1960). In animal cells, morphological changes of the nucleoli are considered to be the most characteristic effects of actinomycin (Goldstein *et al.*, 1960; Journey and Goldstein, 1961, Shoefl, 1964). In this paper results of autoradiographical studies demonstrating the accumulation of actinomycin in cell nuclei are reported.

The actinomycin S_3 used in this experiment was composed of single component which resembled actinomycin B_2 in its paperchromatographical behavior (Kurylowicz, personal communication), although its structure is not fully determined yet. Actinomycin S_3 randomly labelled with tritium by the gas exchange method was prepared by the courtesy of Dr. T. Komai of the National Institute of Health, Tokyo. This preparation of ^{3}H-labelled actinomycin S_3 (^{3}H-ACT) was purified by aluminum column chromatography and then recrystallized from ethyl acetate and finally purified by precipitation with thymus DNA according to the method of Kawamata *et al.* (unpublished). The purified ^{3}H-ACT thus obtained had a specific activity of 30 μc/m mol. By paperchromatography the preparation showed a single spot of the characteristic orange red color of actinomycins. Antibacterial activity and radioactivity on the paper were only demonstrated in this spot. The cells used throughout the experiments were the established strain TG derived from normal human fallopian tube (Barski *et al.*, 1959) which was supplied by Dr. G. Barski, Paris. The cells were grown on a cover slip in a Leighton type square tube containing Eagle HeLa medium (Difco) supplimented with 3.75 % calf serum and 3.75 % horse serum and were fixed with methanol. For the autoradiography, the dipping technique was applied using Kodak emulsion NTB2. After 2 weeks exposure slides were developed and stained with Giemsa solution buffered at pH 5.7. One hundred cells were selected randomly for silver grain counts. When cells were treated with 20 μg/ml of ^{3}H-ACT, the silver grains appeared predominantly in the nuclear region and increased in number with the incubation time. (Fig. 1.) After 2 hours incubation the number of silver grains in the nuclear region had increased markedly, whereas in the cytoplasmic region the number remained unchanged, which indicates the preferential accumulation of ^{3}H-ACT in the nucleus. (Fig. 2.) After cells had been incubated at 4°C with ^{3}H-ACT, no silver grains were detected in any part of the cells. Actinomycinic acid, which is a biologically less active derivative of actinomycin, in which the lactone rings are split off, was obtained by treatment of actinomycin with alkaline

alcohol solution. ^{3}H-actinomycinic S_3 acid was prepared by similar treatment of ^{3}H-ACT. No silver grains were detected by autoradiography after treatment of the cells with this ^{3}H-actinomycinic S_3 acid. The cells, after incubation with 20 μg/ml of ^{3}H-ACT for 2 hours at 37°C, were fixed with methanol and treated with 1 mg/ml of ribonuclease (crystallized, salt free, Worthington) at 40°C for 4 hours or with 50 μg/ml of deoxyribonuclease (crystallized, Worthington) at 37°C for 24 hours. With ribonuclease no loss of silver grains was seen, whereas with deoxyribonuclease most of the grains disappeared.

The present results from autoradiography of ^{3}H-ACT, indicate clearly that, actinomycin binds with cell nuclei. Similar results were obtained with Ehrlich's ascites tumor cells when the cells were incubated *in vitro* with ^{3}H-ACT.

Details of this work will be published elsewhere.

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EXPLANATION OF FIGURES

Fig. 1. Autoradiogram of TG cells treated with ^{3}H-ACT for 60 minutes. The silver grains accumulated predominantly in the nuclear region.

Fig. 2. Autoradiogram of TG cells treated with ^{3}H-ACT for 120 minutes. Intense accumulation of silver grains on the nucleus was found.

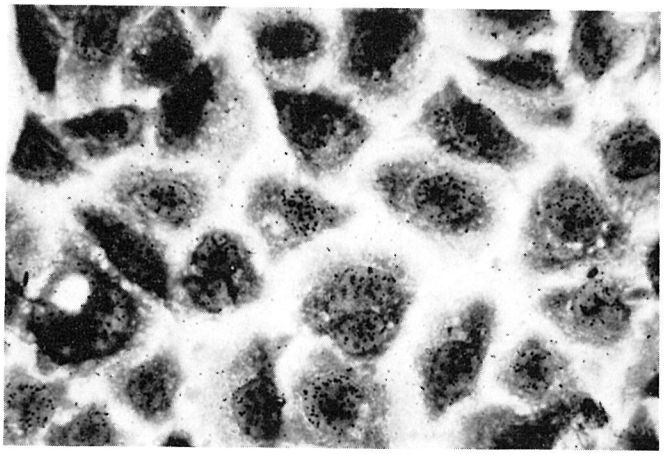

Fig. 1

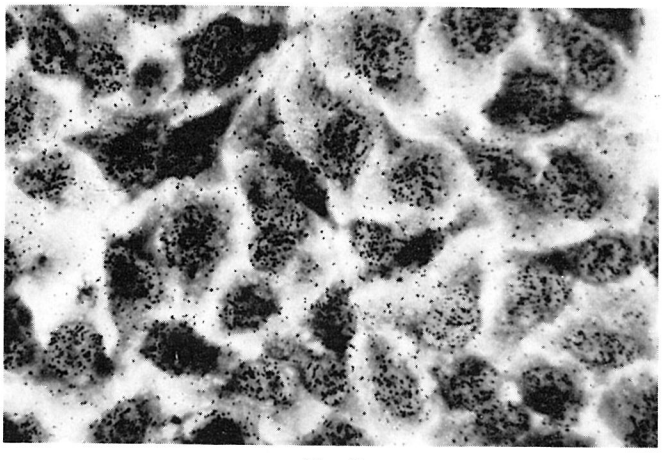

Fig. 2