Letter to the Editors

Detection by Microfluorography (Rapid Scintillation Autoradiography) of Twin Spots Derived from Double Strand DNA Breaks

Atsushi TATARA, Yoshu YOSHIBA and Hikoyuki YAMAGUCHI†

Laboratory of Radiation Genetics and Chemical Mutagenesis, Faculty of Agriculture, The University of Tokyo
1-1, Yayoi 1-chome, Bunkyo-ku, Tokyo 113, Japan

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In order to investigate the chemically or physically produced double strand DNA scissions, we have tried to detect 3'-termini of the lesions. The termini were detected by an enzymic assay using ³H-TTP and terminal deoxynucleotidyl transferase, followed by rapid scintillation autoradiography. This enzyme is generally used for adding complementary homopolymer to vector and cDNA in recombinant DNA technology. As the result, exogenous nucleotides are additionable at random to 3'-hydroxy termini of both single- and double-strand DNA without any templet strand1).

In the present study, barley nuclei were isolated from dry seed embryos2). The nuclear suspension was dropped on a coverglass which was previously coated with a thin layer of gelatin, and the specimens were dried fairly at the room temperature. Then, the dried samples were exposed to a 160 nm vacuum-ultraviolet light at various intensities from the 0.3 GeV electron storage ring at the Synchrotron Radiation Laboratory, University of Tokyo.

After irradiation, the coverglasses turning samples upward were fixed on the slideglasses with polyester resin for convenience of the following treatment.

Reaction mixture for addition of polynucleotide to 3'-termini was consisted of 100 mM sodium cacodylate, 8 mM CaCl₂, 1 mM 2-mercaptoethanol, 0.24% bovine serum albumin, 1.11 MBq (30 µCi)/8 ml, ³H-thymidine triphosphate (³H-TTP; s.a., 832.4 GBq (22.5 Ci)/mM), 400 u/8 ml terminal deoxynucleotidyl transferase (TdT; TAKARA SHUZO Co., Ltd. Japan) and 50 mM phosphate buffer (pH 7.2). The mixture was dropped on the dried samples and incubated for 2 h at 25 °C. The incubation temperature was kept at 25 °C for restricting continuous addition of exogenous nucleotides towards 3'-hydroxy termini of DNA strand breaks. After 2 h-incubation, the mixture was washed away with 9% NaCl and the reaction was stopped by soaking into ice-cold 5% TCA for 30 min. Then, the specimens were thoroughly washed with distilled water and dried at room temperature.

After staining with Feulgen's reagent, the dried samples were subjected to microfluorography (rapid scintillation autoradiography). The method has been reported previously by Yamaguchi and Tatara in 19843). We proposed in this paper that dioxane scintillator was available for the detection of tritium incorporation into samples.

The results were shown in Fig. 1. We could...
Fig. 1 Twin spot of silver grains observed on each nucleus.
These samples were exposed to 29.6 V min of an 160 nm vacuum-
UV light with the dose rate of 0.11 V (100 mA)^−1 min^−1.

find twin spot of grains closed by on a nucleus in the photographs as seen in Fig. 1. However, we could find out no such twin grains on the slide of unirradiated control, as well as on the slide after using DNA polymerase I and ^3^H-TTP.

Consequently, this method is available for cytological detection of radiation-induced double strand DNA breaks.

References