DNA-REPAIR DEFICIENT CELLS IDENTIFICATION WITH A MULTIWIRE PROPORTIONAL CHAMBER ☆

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Received 30 June 1982

Tritium labelled mammalian cells with defective repair of UV-induced damage have been identified by using a MWPC as a position sensitive radioactivity detector. The resolving power ($\simeq$1.5 mm FWHM), sensitivity ($\simeq 10^{-1}$ Bq/cm^2), efficiency ($\simeq$10%) and uniformity ($\simeq$4%) of the detection system are shown and "electronic autoradiograms" of normal and mutant cultures are presented; cells, rescued after the radioactivity measurement, retain their cloning ability.

The preservation of structural integrity of genetic material is a necessary requirement for its function. Fundamental importance is therefore attached to those biological processes which are needed for the repair of damage induced on DNA by physical or chemical agents. For this reason, a number of investigators have attempted the isolation of repair – deficient mutants from in vitro cultures of human or other mammalian cells [1–4]. In previous papers [5,6], we proposed a new method for the isolation of DNA repair-deficient mutants and we showed that "electronic autoradiograms" of living, repairing cells can be succesfully obtained. The method relies on the differential incorporation of radioactive precursor of DNA biosynthesis in mammalian cells damaged by UV-irradiation and on a specially designed Multiwire Proportional Chamber (MWPC) that acts as a position sensitive radioactivity detector. Using ^{14}C-thymidine (E_{max} of the ^{14}C β-rays = 156 keV) as the labelling agent we initially obtained a resolving power of 4–5 mm (FWHM) and a sensitivity $<$3 Bq/cm^2. With these figures it is possible to reduce the acquisition time from the several weeks necessary for conventional autoradiography to a few hours. To further reduce the running time of the experiment – critical for the cell survival in a hostile environment – and to improve the sensitivity and resolving power of the method, we have explored the possibility of using ^{3}H-thymidine as the labelling agent. This compound can be easily obtained with specific activity 1000 times higher than ^{14}C-thymidine.

However, the very low energy (E_{max} = 18 keV) of the ^{3}H β-rays, which correspond to a maximum range of 0.59 mg/cm^2 ($\simeq$4 μm in mylar or $\simeq$3 mm in argon STP), requires practically no absorbing material between the samples and the active volume of the detector. This requirement has been satisfied by replacing the mylar window of the MWPC with an easily removable top frame. In this way the samples can be easily placed inside and removed from the detector. On the other hand, the very short range of the ^{3}H β-rays improves the resolving power of the system to $\simeq$1.5 mm (FWHM) and the reduced spread-out of a point-like source at the detection plane increases the sensitivity ($S \simeq 10^{-1}$ Bq/cm^2). The overall ^{3}H detection efficiency has been measured to be $\simeq$10% and the

☆ This work has been partially supported by P.F. "Controllo della Crescita Neoplastica" of CNR.

0 031-9163/82/0000–0000/$02.75

uniformity of response to a two-dimensional activity distribution has been checked by moving a ^3H disk source of 5 cm^2 area at about forty different positions inside the chamber. The standard deviation of the count distribution is 4% when the data are integrated over a 5 mm^2 area.

A data acquisition and processing system with both analog and digital capability has been implemented. The analog branch is conveniently used for trouble shooting as well as for control and immediate feedback on the experiment, whilst the digital one is used for off-line data reduction and analysis.

In order to test the ability of the MWPC to discriminate between mutant and normal colonies, three reconstruction experiments were performed.

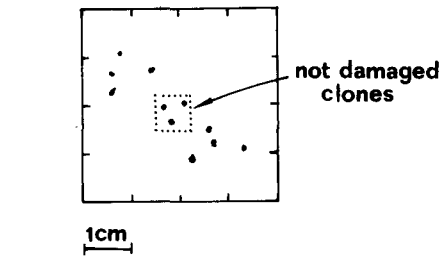

Fig. 1. (a) A pattern of several clones of ^3H-labelled cells. (b) The reconstructed map of the cell pattern: (1) original image, (2) the same of (1) after background subtraction, (3) the contours of the clones at 30% threshold, (4) the contours of the *not damaged* clones.

In the first experiment colonies of the human established cell line HeLa were grown on glass slides, UV-irradiated, and labelled with ^3H-thymidine in the presence of hydroxyurea. Since DNA replication is almost completely blocked by the inhibitor, the radioactive precursor will mainly be utilized by the cells engaged in the repair of DNA sequences damaged by the UV-treatment (see refs. [5,7]). Slides containing both irradiated and unirradiated clones were simultaneously analyzed by the MWPC. The amount of radioactivity taken up by unirradiated colonies (with undamaged DNA) should be similar to that incorporated by mutant colonies (unable to repair the UV-induced DNA damage). As shown in fig. 1 the mutant mimicking, unirradiated colonies, are well discriminated from the irradiated (repairing) colonies. By means of a simple off-line analysis, it is also possible to improve the S/N and to quantitatively assess the mean count density for both irradiated and non-irradiated cells. The overall acquisition time is only a few minutes ($\simeq$15), including the time spent to restore the working conditions after opening the MWPC. After the radioactivity measurement, the HeLa cells have been rescued from the detector and further cultured; they have shown retention of their cloning ability.

In a second experiment we used cells of uvs-20, a UV-sensitive mutant isolated from the EUE human cell line. The ability of uvs-20 to perform DNA repair synthesis is about 55% of the parental cell line [8].

Fig. 2. MWPC image of uvs-20 and EUE cell monolayers showing: (1) the original image, (2) smoothing + background subtraction, (3) the contours of monolayers with a 10% threshold.

Cellular monolayers of both EUE and uvs-20 were UV-irradiated, labelled and analyzed with the MWPC together with three unirradiated layers that constitute a common background reference (fig. 2). The ratio of the mean count density between uvs-20 and EUE cells has been measured to be $\simeq 0.5$.

Finally, the ability of the MWPC to discriminate xeroderma pigmentosum cells, a well known human cell type with a severely impaired excision repair [9], from normal human cells was tested. Human diploid fibroblasts and XP fibroblasts were grown on separate glass slides, irradiated, and labelled. The result of the analog processing of these cells is shown in fig. 3. As one can see, the XP cells, completely unable to repair their damaged DNA give out no detectable signal, whilst the NF cells are clearly imaged.

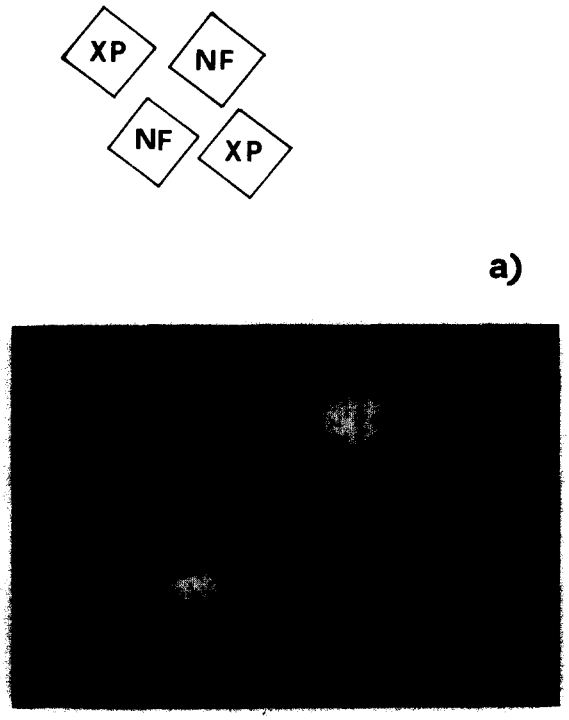

Fig. 3. (a) A pattern of cellular monolayers of XP and normal human fibroblasts (NF) (b) Analog processed image of the pattern shown in (a).

From these experiments we can conclude that the MWPC allows to discriminate and to precisely locate cell types having or lacking the ability to repair DNA damage.

It should then be possible to isolate repair-deficient mutants from cultured human cells by simply screening for lack of ^{3}H-incorporation in hydroxyurea-blocked, UV-irradiated colonies.

Although a very specific use of the MWPC has been described here, many other applications can be envisaged whenever variations in the ability of cell clones or other cell aggregations to incorporate or attract radioactive precursor have to be studied. The MWPC, a device initially developed for high energy physics experiments can then be a very useful tool in radiation biophysics too.

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