no difference in the gene copy numbers between LY sublines.

As shown in the Figure, after X-irradiation, RT-PCR analysis pointed to only a slight transient increase (at 15 min) in DNA-PKcs and Ku70 mRNA in LY-R cells. LY-S cells responded with similar transient expression of DNA-PKcs mRNA, however, they showed a marked, continuous increase in Ku70 and Ku80 mRNA levels until 60 min after irradiation. So, the DSB repair defect in LY-S cells is not related to absence or insufficient expression of genes coding the components of the DNA-PK complex.

X-irradiation of mammalian cells induces potentially lethal damage (PLD), a sector of which is sensitive to treatment with hypertonic salt solution, usually 0.5M NaCl. Radiosensitisation by such treatment has recently been explained [1]. In rat fibroblasts 80% of the Ku subunits of the DNA-dependent protein kinase (DNA-PK), component of double strand break (DSB) repair system, non-homologous end-joining (NHEJ), are localized in the cytoplasm. Upon irradiation, there is a translocation of these proteins to the nucleus. Altered toxicity of the medium inhibits the translocation, thus impairing the fast DSB repair and, in consequence, diminishing survival. Increase in nuclear Ku subunits was not observed in fibroblasts from a mutant strain of Long-Evans Cinnamon (LEC) rat that has an enhanced radiosensitivity and a reduced level of repair of DSBs after X-irradiation [2] – in contrast, 10 min after irradiation a loss of Ku from the nucleus was observed. This was in spite of the presence of all subunits of DNA-PK identical with the wild type rat cells. Therefore, a defect in maintaining the levels of Ku subunits in the nuclei of LEC rat cells was suggested.

The properties of LEC rat cells are reminiscent of those of LY-S cells, a radiation sensitive subline of LS178Y murine lymphoma. These cells contain DNA-PK activity comparable to that of the parental, radiation resistant LY-R cells, when measured in vitro [3], but exhibit a defect in DSB repair, most pronounced in G1-phase of the cell cycle [4], apparently due to impaired NHEJ [5]. We checked, whether hypertonic salt solution affects the response to X-irradiation of LY-S cells, in view of the possibility that a similar defect as in LEC rat cells is the cause of their radiation sensitivity. LY-R cells served as a reference strain. The cells were X-irradiated with 2 Gy at +4°C in cell culture medium and NaCl solution added to the final concentration of 0.5M (identical volume of isotonic salt solution added to the controls). The cells were then placed at 37°C and, after 20 min, bovine serum added to 28% concentration (in order to prevent cell lysis during centrifugation, that is necessary for medium replacement). After 48 h incubation in the full culture medium relative cell numbers were estimated.

The results in Fig. show that LY-R cells are sensitised by the treatment, whereas LY-S cells are not. This indicates that in contrast with LY-S cells, the fast PLD repair in LY-R cells is inhibited in hypertonic medium in a manner comparable to that in wild type rat cells [1]. Thus, the effect of hypertonicity may rely on translocation of Ku subunits, as proposed by Endoh et al. [1]. We wanted to confirm these conclusions by comparing the amounts of Ku70 in the cytoplasmic and nuclear extracts in control and X-irradiated cells, with the use of Western blotting. The expected difference was found in LY-R cells (increase in nuclear Ku70, 10 and 60 min after irradiation, with a concomitant decrease in the cytoplasmic Ku70 (not shown). In contrast, in LY-S cells no changes in the levels of nuclear and cytoplasmic Ku70 were found. So, the radiation sensitivity-related defect in LY-S cells is different from that in LEC rat cells.

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References
References


A CROSS-PLATFORM PUBLIC DOMAIN PC IMAGE-ANALYSIS PROGRAM FOR THE COMET ASSAY

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The single-cell gel electrophoresis (SCGE), also known as the comet assay, is a sensitive method to measure genotoxicity and cytotoxicity of chemical and physical agents [1-3]. SCGE has also been used to analyse the capacity of cellular DNA repair [4-6]. Although the methodology of SCGE is straightforward and does not require sophisticated equipment, the analysis of comet images is not so simple. The visual classification of comets on the basis of their morphology and degree of damage is possible, but is not very precise [3]. A better sensitivity is achieved by computer analysis of comet images. Several commercial applications are available, however, they are generally sold as combined soft-

Fig. View of the program windows. In the left-hand window the comet image with the measurement frame is visible, with tail and head marked. The measurement frame is split into two frames: a background and a comet frame. In the right-hand window the intensity profiles are plotted and the checkbox bar is shown with options of profile and image view. Below the profile window selected measurement results are printed.