

ated LY cells, we found no difference in the drug's effect on the post-irradiation cell kill.

## References

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## TOTAL TYROSINE PROTEIN KINASE ACTIVITY IN X-IRRADIATED L5178Y CELLS

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Tyrosine protein kinase (TPK) activity is manifested by 2 classes of enzymes: receptor kinases (e.g. epidermal growth factor receptor, EGFR) and non-receptor kinases (among them many proto-oncogenes). Both transfer a phosphate residue from a nucleotide, usually ATP, to a substrate protein. This results in an alteration of the protein's biological activity; the consequence is the modulation of the relevant cellular process (review in [1, 2]); TPK activity is essential in the cell cycle control, cell-cell communication, generation of lipid-linked second messengers etc. Its importance for the cellular response to ionising radiation has been studied for the last few years.

TPK activation is a mandatory proximal step in radiation-induced signalling through protein kinase C-dependent pathway [3]; some further steps downstream there are such important tyrosine-phosphorylated kinases as RAF1 and MAPK (mitogen activated protein kinase). Also the c-Abl TPK becomes activated in irradiated cells and plays an important role in the cellular response, both in cell cycle progression and radiation-induced apoptosis (review in [4]).

For determination of TPK approximately  $4 \times 10^7$  LY-R or LY-S cells were X-irradiated (1 Gy) and the whole cell extracts were prepared according to Finnie et al. [5] after time intervals indicated in Fig.1. Total TPK activity was determined in cellular extracts with the use of Boehringer-Mannheim (GFR) ELISA kit, according to the manufacturer's procedure.

TPK activity in the control and X-irradiated LY cells was estimated with the use of two protein kinase substrates, PKS1 and PKS2 (Fig.1 legend). Figs.1A and B show the results obtained with PKS1 and PKS2, respectively. In the control LY-R cells, the activity was the same with both substrates and the course of TPK activity increase and decrease - almost identical. In the control LY-S cells, the activity was lower than that in LY-R cells with PKS1 but higher - with PKS2. The differential substrate specificity of TPK in the LY sublines also was seen after irradiation: with PKS1 stimulation, it was delayed in LY-S, as compared to LY-R cells, but was of similar magnitude; with PKS2 stimulation, it was seen 1 h after irradiation, but again, the highest activity in LY-S cells was revealed 3 h after irradiation, when it exceeded that in LY-R cells. At that time point, the TPK activity in LY-R cells returned to the control level. So, in spite of the

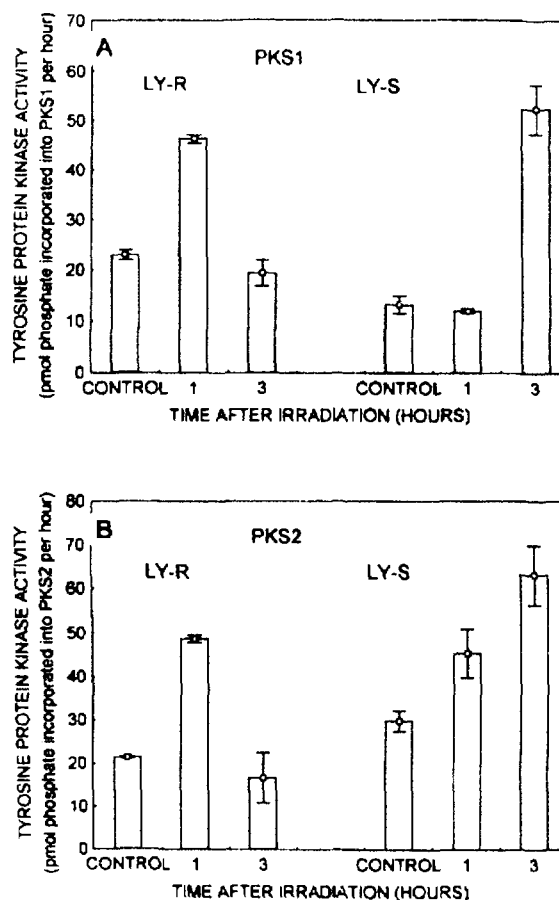


Fig.1. Stimulation of tyrosine protein kinase activity by X-irradiation in LY-R and LY-S cells. A) PKS1 - protein kinase substrate 1 (Biotin-KVEKIGEGT<sup>1</sup>YGVVK-amide) corresponds to amino acids 6-20 of the cell division kinase p34<sup>cdc2</sup>, B) PKS2 - protein kinase substrate (Biotin-EGPWLEEEEFAYGWMDF-amide) corresponds to the amino acid sequence 1-17 of gastrin. Cells were X-irradiated (1 Gy); after 1 or 3 h incubation whole cell extracts were prepared according to Finnie et al. [5]. Data shown are mean results of 2 determinations  $\pm$  range.

close relation of the LY sublines and many similarities in the basic characteristic features [6], the pattern of TPK activation in the X-irradiated LY sublines differs; this observation warrants further investigation of PTK in these sublines.

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## APOPTOSIS IN MAMMALIAN CELL LINES WITH DIFFERENT RADIATION SENSITIVITY: COMPARISON AFTER LOW DOSE X-IRRADIATION

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In the past few years the interest for using apoptosis as a possible measure of radiosensitivity has been substantially increased both with regard to the possibilities of using the extent of apoptosis as a biological dosimeter [1] and for estimating the radiosensitivity of cancer cells prior to radiotherapy [2-4].

In this work we have measured apoptosis in 5 relatively radiation sensitive cell lines following X-ray irradiation with doses from 0.1 to 2 Gy. These were two lymphoid AT cell lines, one homozygous and one heterozygous for the ATM gene, the human pre-B cell line, Reh, and two murine L5178Y lymphoma cell lines, LY-R and LY-S.

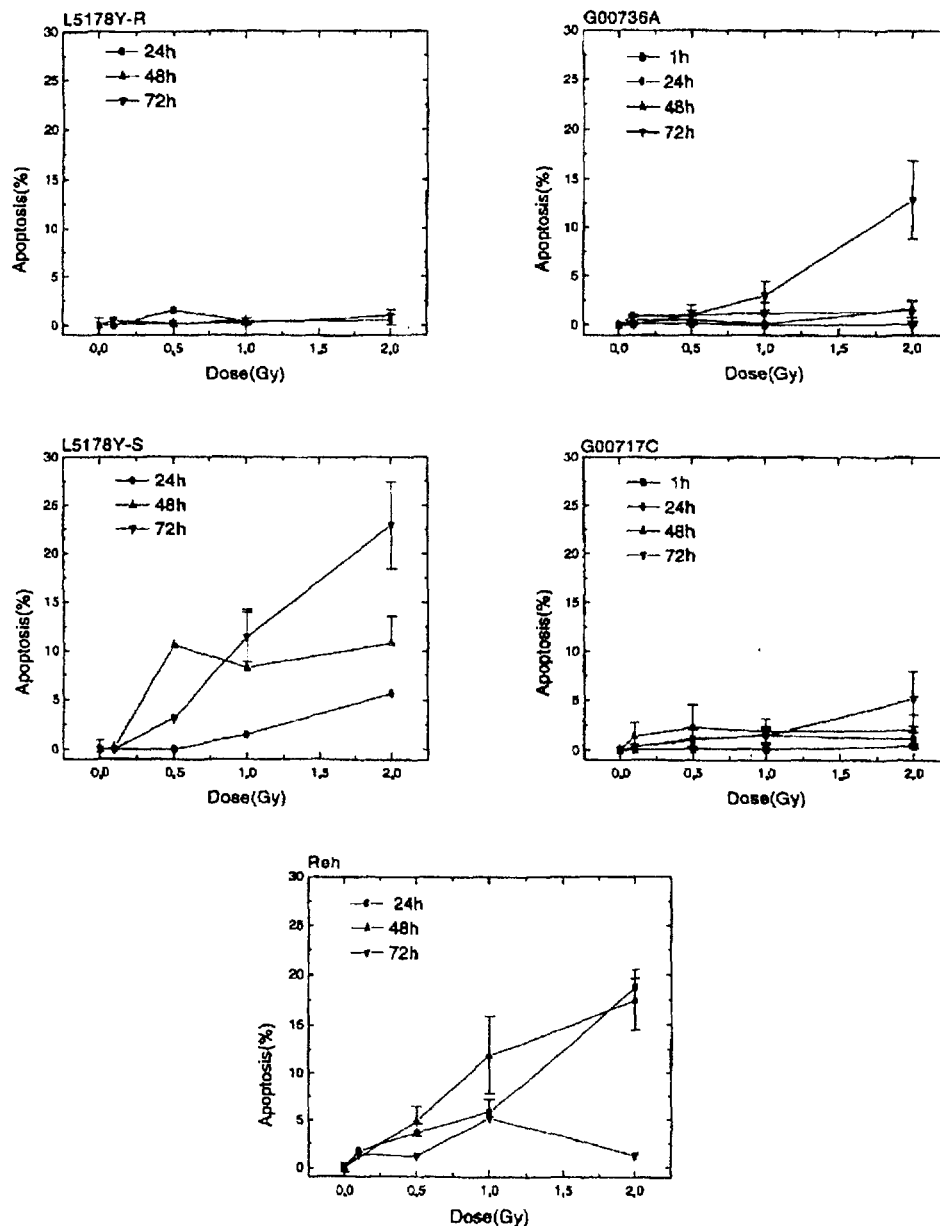


Fig. X-ray-induced apoptosis in human and murine lymphoid cell lines, estimated by the TUNEL method.