6 SUMMARY

Detection of UV-induced gene activation in mammalian cells by means of a GFP vector stably integrated into the genome

In order to benefit from the advantages of the cofactor-independent expression of "Green Fluorescent Protein" (GFP) in heterologous systems and its detection in living cells for the exploration of the effects of ultraviolet (UV) radiation in mammalian cells, a stable cell line was established, by means of which UV induced gene activation can be measured as green fluorescence of the reporter protein "Enhanced GFP" (EGFP).

For this purpose, the suitability of EGFP and its destabilized variant, d2EGFP, as reporter of promoter activity in mammalian cells was examined. By means of stably transfected, constitutively EGFP or d2EGFP expressing CHO cells, different detection methods were tested. Compared to the parental cell lines, these stably transfected cell lines showed no change in growth behaviour and in the sensitivity towards X-rays and UVC radiation. EGFP was either monitored in living and in formaldehyde fixed cells by FACS analysis and in the fluorescence microscope in each individual cell, or by a time-saving procedure using a microplate reader. A linear correlation between fluorescence intensity and cell number per well was found. Using these stably transfected cells, the effect of cytotoxic agents on cell growth can be measured as a reduced EGFP fluorescence increase in the microplate reader. Constitutively expressed d2EGFP, which shows a half-life of 3 h in CHO cells, can only be monitored by FACS analysis and in the fluorescence microscope, but not in the microplate reader.

For the examination of UV induced gene expression, the stably transfected cell line HEK-pNF-κB/Neo was generated, in which the reporter gene d2EGFP is under the control of a synthetic promoter, which consists of four NF-κB binding sites and the minimal thymidin kinase promoter. In this human embryonic kidney (HEK) cell line, UVA induced gene expression can be measured as an increase of EGFP fluorescence. TNF-α treatment of cells gave rise to substantial d2EGFP expression in up to 90 % of the cells and was therefore used as a positive control of induction of NF-κB dependent gene expression. UVC and UVB radiation caused no increase in d2EGFP expression, X-rays did so only at high doses. Treatment of cells with the tumour promoter PMA produced d2EGFP expression in up to 40 % of the population. The detection of this induced d2EGFP expression required FACS analysis or inspection in the fluorescence microscope, measurement in the microplate reader was impossible due to the low fluorescence intensity.