Expression of GFP Tagged Proteins in Mammalian Cells

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The green fluorescent protein (GFP) contains a chromophore domain, which emits light upon excitation with light of the appropriate wavelength. Molecular biological techniques enable researchers to couple the gene coding the GFP with that of a protein of interest (POI) which leads to the expression of a GFP-POI fusion protein in cells. Here the POIs are DNA repair proteins, which accumulate in large amounts at the site of DNA damage, induced by ion irradiation. The fusion proteins can be observed and traced in living cells using live cell fluorescence microscopy techniques [1].

For the expression of any protein in a cell, a regulatory element in front of the gene, the promoter, is necessary for the initiation of the transcription. Transcription is the process of copying the genetic information of the DNA to a "mobile information carrier", the messenger RNA (mRNA), which is essential for the protein synthesis at the ribosomes, meaning that the minimal DNA unit would consist of a promoter-gene construct. But in practice larger circular units, so called plasmids or vectors are used to introduce genetic material into cells (transfection). Plasmids usually contain a gene for bacterial antibiotic resistance, for easily amplifying the vector in bacteria and a gene for mammalian antibiotic resistance to easily select those cells harbouring the plasmid. Normally the gene for resistance and the gene for the GFP-POI fusion protein are regulated by separate viral promoters. Viral promoters are used because they lead to high transcription levels, at least for a short time. In contrary, during long term handling of transfected cells, viral promoters often are silenced, "switched off", by the cellular virus defence machinery. This leads either to cell death, if the resistance gene is silenced or to antibiotic resistant cells without GFP-POI fusion protein expression taking over the population. A long term handling is necessary if the transfection rate is low or if well characterized single cell clones, e. g. regarding the expression level of the fusion protein, will be established.

To prevent the cells from quitting the expression of the GFP-POI fusion protein a new vector generation has been developed. These take advantage of internal ribosome entry sites (IRES). An IRES element allows the ribosomes to use an additional start signal for protein synthesis on the mRNA. This results in the synthesis of two different proteins coded on a single mRNA molecule (dicistronic), in our case the GFP-POI fusion protein and the protein conferring the antibiotic resistance. Since a single mRNA is controlled by only one promoter, silencing the promoter of the fusion protein will lead to cell death due to loss of antibiotic resistance.

For experiments at the live cell imaging facility of the ion microprobe SNAKE we decided to reclone our existing GFP-POI fusion proteins into this new dicistronic vector generation. Up to now, a 53BP1-GFP expression construct has been finished and cells transfected with this construct have been successfully irradiated and monitored online at the facility (see figure 1). Work to reclone MDC1-GFP is under progress and Rad52-GFP will be started this year.

References

[1] V. Hable *et al.*, this report 74.

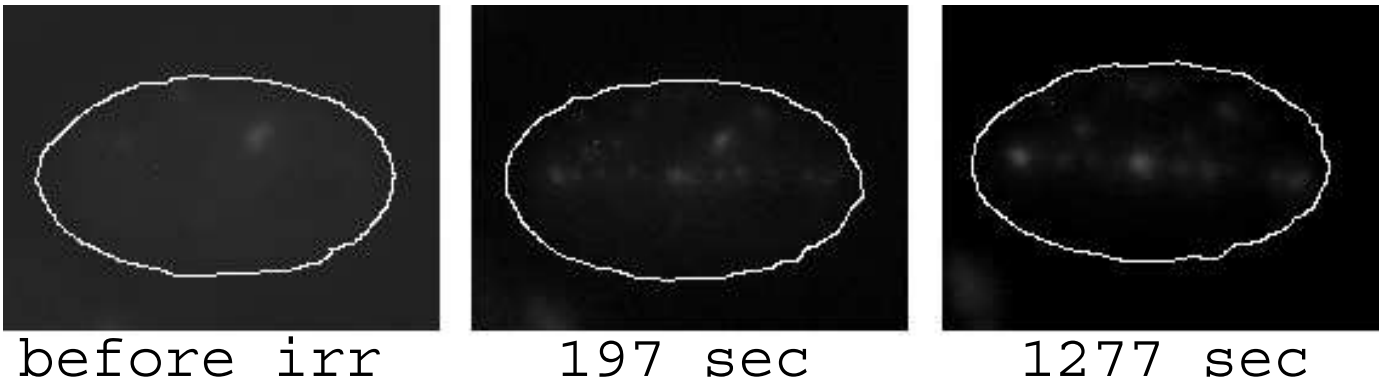

Fig. 1: Hela cell (nucleus marked by a white fringe) stably transfected with the dicistronic vector pMC16-53BP1-GFP has been irradiated at the live cell imaging facility of the ion microprobe SNAKE with 55 MeV Carbon ions in a line pattern ($\Delta x = 1.1\mu m, \Delta y = 4.9\mu m$) and monitored online for 1277 sec. Ion traversal through the nucleus causes DNA damage, at which the protein 53BP1-GFP accumulates and gives rise to brighter signals.