

## Background

Measuring gene expression using microarrays is relevant to many areas of biology and medicine, such as follow up of developmental stages and diseases onset, and treatment study. Since there can be tens of thousands of distinct probes on an array, each microarray experiment can accomplish the equivalent number of genetic tests in parallel. Arrays have therefore dramatically accelerated many types of investigations. For example, microarrays can be used to identify stress response genes by comparing gene expression in challenged versus normal cells. In the Molecular and Cellular Biology lab (MCB), the microarray experiments are performed within the Genomic Platform, fully equipped to analyse either the behaviour of bacteria during long space flight, the effect of low dose ionising radiation on the developing organism in mice, or the human individual radiation sensitivity.



*Microarray process as performed in the Genomic platform of the Molecular & Cellular Laboratory. Glass slides are spotted with unique DNA sequences, each corresponding to a unique gene to end with an array as small as a microscope slide containing up to 40.000 dots (gene), called microarray. The array is then hybridized with expression material "RNA" purified from living cells and labelled in vitro with fluorescent dyes (green for the control condition and red for the treated one). Laser scanning of the slide after hybridization allow to quantify the amount of green and red labelled RNA hybridized to each spot on the array. Statistical analysis will determine the ratio of up and down regulated genes.*

## Objectives

For the low dose effect, two main stages of development are of interest; 1) the gastrula stage at which ionizing radiation can induce several malformations. 2) the organogenesis. During brain development, epidemiological studies of the atomic bomb survivors of Hiroshima/Nagasaki showed increased risk of mental retardation in children of women exposed between weeks 8-15 of pregnancy or at a lower extend between weeks 15-25.

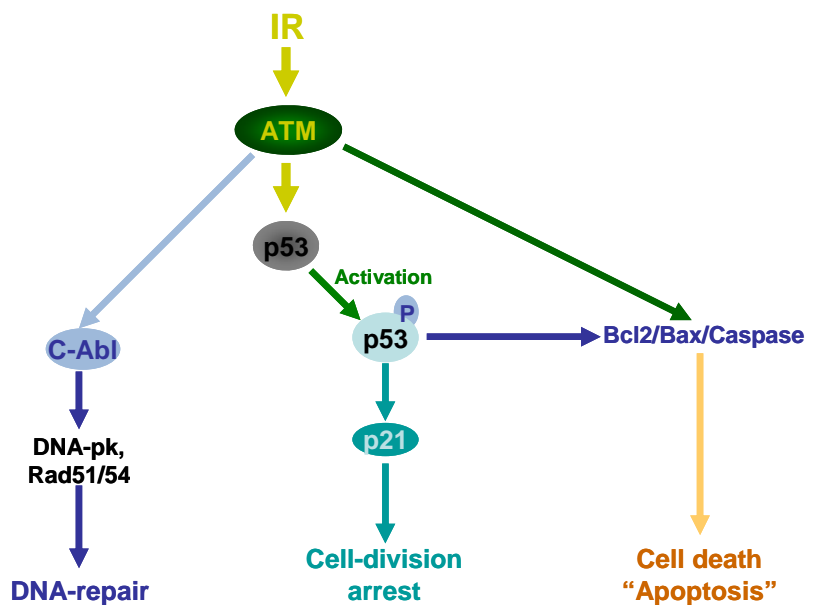
## Principal results

1) Gastrulation in mice is associated with extreme cell proliferation and differentiation. Exposure to ionising radiation during this stage of development leads to a high production of damaged cells. Gene expression analysis have been performed in our laboratory using the microarray technology to analyse the differential expression induced 2 h after 50 cGy whole body irradiation of the embryonic and extraembryonic parts of the gastrula. As it has been demonstrated *in vivo*, the gastrula is hypersensitive to DNA damage induced by low dose irradiation (<0.5 Gy) and undergoes apoptosis without cell cycle arrest. Indeed gene expression profile confirm the observations done by other groups, ATM is upregulated 4.6 times in the embryonic part and only 1.2 times in the extraembryonic region. As ATM, several other apoptotic and p53 downstream regulating genes are induced suggesting elimination of some damaged cells by apoptosis.

Surprisingly cell cycle progression transcripts are upregulated in the embryonic part suggesting that no cell cycle arrest occurs prior apoptosis. In this study we demonstrate at the molecular level a novel surveillance mechanism for the elimination of cells damaged by ionizing radiation during mouse gastrulation, while non compromised cells continue their proliferation.

2) Brain damage induced by prenatal irradiation is of major concern in radiation protection. Using cDNA-microarrays and real-time PCR we analyzed the modulated genes in the 50 cGy X-ray irradiated embryonic mouse brain. The main activated pathways are the *Trp53* dependent programmed cell death, and the intercellular signaling cascades. The strong upregulation of *Ccng1*, *Trp53inp1* and *Cdkn1a* suggested that the tumour suppressor P53 is an essential regulator of the radiation induced stress response (fig2). Although in the *Trp53* null mutant embryos, our data highlights a generalized down-regulation of genes involved in cell cycle progression.

At the cellular level, beside cell death and cell cycle arrest, we analyzed the neurite outgrowth by measuring the length of the neurites extruding from the differentiating neurons. Analysis of neurite extension resulted in a statistical significant reduction for both 50 and 100 cGy X-ray exposure. Taken together, radiation induced cell death of astrocytes in the cerebral cortex, and reduction in neurite length in maturing neurons, may interfere with a correct patterning of the brain and could jeopardize the formation of a correct neural network, leading to cognitive deficits in the mature brain.



*Transcriptional cascade of regulated genes after radiation exposure. Solid lines depict mechanisms shown in the developing organism. Ionising radiation "IR" induces the ATM gene that in its turn induces the p53 gene (responsible for the integrity of the genome). In the developing brain p53 induces cell death in compromised cells, and stop cell division in cells affected with damage in the genetic material (DNA). When the damage is severe and the DNA could not be repaired, the cell dye, otherwise repair mechanisms are activated to repair the damage*

#### Future perspectives:

- 1) In the framework of the EC project "NOTE" and the FANC project, studies on the mouse gastrula will be followed for lower irradiation doses and for different strains mutated in key genes as shown in the figure .
- 2) Behaviour studies are actually performed in collaboration with (Prof. P. De Deyn, UZA, Antwerp). Molecular analysis of neurons of behavioural defective mice will help to make a link between the damage and its phenotypical expression after exposure to 0.5Gy x-rays.

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#### Main references:

Verheyde J, Benotmane MA. Unraveling the fundamental molecular mechanisms of morphological and cognitive defects in the irradiated brain. *Brain Res Brain Res Rev.* 2006 Dec 21; [Epub ahead of print]