

Background

The human body contains approximately 10^{14} cells, wherein each one is a nucleus. The nucleus contains 2x23 chromosomes, or two complete sets of the human genome, one set coming from the mother and the other from the father. In principle each set includes 30.000-40.000 genes. If the genome was a book, it would be twenty-three chapters, called chromosomes, each chapter with several thousand stories, called genes. Each story made up of paragraphs, called exons and introns. Each paragraph made up of 3 letter words, called codons. Each word is written with letters called bases (AGCT). But the whole is written in a single very long sentence, which is the DNA molecule or deoxy nucleic acid. The usual state of DNA is two complementary strands intertwined forming a double helix. In the cell, DNA is duplicated during each cell division to ensure the transmission of the genome to the daughter cells. For expression, the DNA is transcribed to messenger RNA. The RNA is edited and finally translated to a protein, each three bases coding for one amino acid. When the whole message is translated, the chain of amino acids folds itself up into a distinctive shape that depends on its sequence. Proteins are the effectors of the genes, and are responsible for all metabolic, hormonal and enzymatic reactions in the cells. The expressed RNA determines the amount of proteins to be produced and subsequently the desired effect (strong or weak) in the cell.

The microarray technology aims at quantifying the amount of RNA present in the cell from each expressed gene, and at evaluating the changes of these amounts after exposure of the cell to toxic chemicals, ionising radiation or other stress components. The global picture of expressed genes helps to understand the affected genetic pathways in the cell at the molecular level. The microarray technology is used in the Radiobiology & Microbiology topics to study the effect of ionising radiation on human cells (FigB) and mouse tissue, as well as the effect of harsh environment and space conditions on micro-organisms.

Microarray spotting

Consists of depositing an amount of nucleic acid, protein or antibody using high precision spotting pins capable of creating 100µm diameter spots on precoated glass slides as horizontal support. Performed with a high precision spotting device (MicroGrid), using 48 spotting pins and capable of printing 1.000 single strand DNA molecules (probes) on 100 precoated glass slides in 1 hour time. Each spotting pin is able to print an area of 24 x 24 spots, which means 576 spots per pin and a total of 27.648 spots in 10cm square area on the slide using 48 pins (FigA). Each spot corresponds originally to the sequence of one specific gene or probe. After spotting, the DNA is cross-linked to the glass slide by exposure to UV light.

Experimental set-up & hybridisation

The aim of microarray experiments is to compare in a quantitative way the amount of RNA from each expressed gene between two experimental conditions. The two expressed RNAs are then used to produce differentially labelled complementary DNA during reverse transcription procedure. This labelling is performed with fluorescent dye labelled nucleotides. In our case we use the Cy3dCTP which gives a green colour with the control condition and the Cy5dCTP which gives a red colour with the experimental condition (FigA). The remarkable ability of nucleic acids to form duplex from two strands with complementary base sequences is the basic microarray. The mix of the differentially labelled cDNAs (control Cy3 and treated Cy5) is then deversed on the top of the spotted glass slide.

Microarray scanning and analysis

Fluorescence measurements at the respective emission values of Cy3 and Cy5 (532nm and 635nm) is performed by a laser scanner. The shape and morphology of the spots is analysed after scanning.

Quantification of fluorescence values is combined with deep statistical analysis to determine the ratios of up and down regulated genes. Modulated genes with similar expression patterns are then clustered in metabolic pathways.

Principal applications

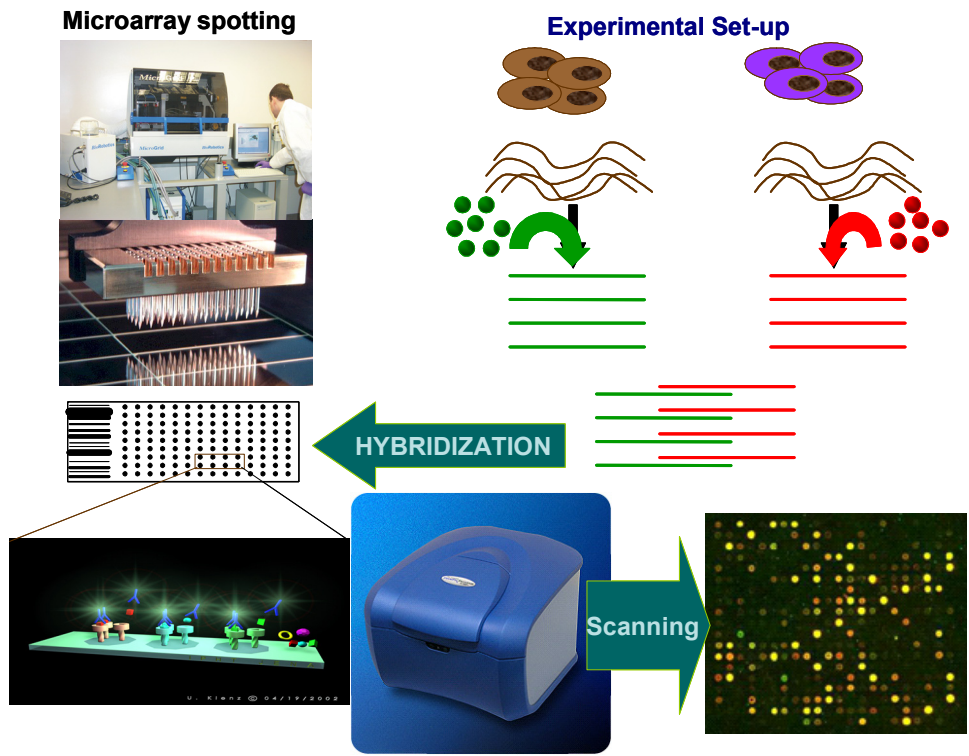
The genomic platform has been financed mainly via space projects from the European Space Agency (ESA) to study the effect of microgravity and cosmic radiation on the behaviour of living micro-organisms such as *Cupriavidus metallidurans* in space conditions. The technology is used also in radiobiology for the investigation of the effects of ionising radiation on the molecular level.

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Main reference

F. Thierry, M.A. Benotmane, C. Demeret, M. Mori, S. Teissier, C. Desaintes, *A genomic approach reveals a novel mitotic pathway in papillomavirus carcinogenesis*. Cancer Res. 2004 Feb 1;64(3):895-903.



The microarray process from microarray spotting, hybridisation with experimental conditions and laser scanning of the spots on the glass slide.

