

## Commentary

# DNA hybridization arrays: a powerful technology for nutritional and obesity research

Obesity is a serious and growing public health problem throughout much of the world. In fact, obesity has reached epidemic proportions in many developed countries. Increased food intake and decreased energy expenditure due to the sedentary lifestyle of our developed societies have contributed to the widespread development of obesity. However, these environmental factors cannot totally explain the development of the disorder. In fact, genetic predisposition for obesity may underlie the increased tendency for weight gain in some individuals (Levin, 2000; Marti *et al.* 2000). Recent research has identified over 200 genes, markers and chromosomal regions associated with or linked to human obesity (Chagnon *et al.* 2000). The contribution of genetic background to obese phenotypes is a complex question yet to be answered. The major aim, therefore, is to study those combinations of genes and mutations that are implicated in the development of obesity in human subjects. In order to be able to control the spread of obesity, it is necessary to establish how environmental factors affect the onset of obesity in individuals with a specific genetic background.

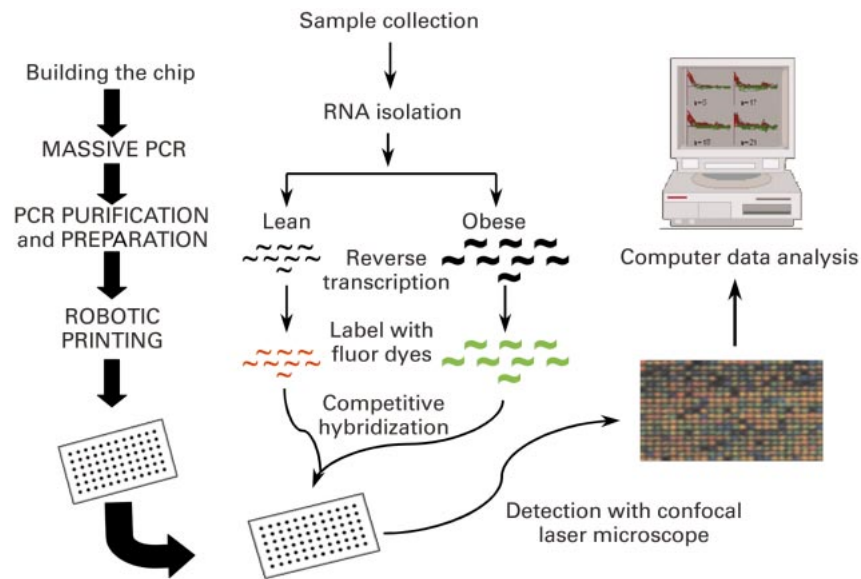
With potentially thousands of genes examined in a single hybridization, DNA hybridization array technology constitutes a powerful tool to identify and compare patterns of gene expression (Lander, 1999; Yang *et al.* 1999). Thousands of DNA spots are robotically deposited on a solid-state matrix (nylon membranes, glass microscope slides, silicon or ceramic chips). These matrixes are then hybridized with labelled cDNA representing total RNA pools from test and reference samples. The differential expression of genes between both test and reference samples are detected based on the fact that the higher the expression of a gene, the higher the intensity of the output labelled signal (Schena *et al.* 1995, 1996; Duggan *et al.* 1999).

An array starts with the selection of the 'probes' to be printed in the array. In many cases these probes are chosen directly from databases like Genbank (Benson *et al.* 1998), dbEST (Boguski *et al.* 1993) and UniGene (Schuler *et al.* 1996), the resource backbones of the array technologies (Duggan *et al.* 1999). There are a variety of options for making arrays (Bowtell, 1999; Cheung *et al.* 1999), with four different array types developed according to the different characteristics of the matrix, probe number and density, array size and type of label. These include macroarrays, microarrays, high-density oligonucleotide arrays and microelectronic arrays (Freeman *et al.* 2000). In the macroarrays the DNA probes are immobilized on a membrane-based matrix, the targets are radioactively labelled, and the different samples are hybridized on

individual separate arrays. Phosphoimagers are then used to detect the amount of bound labelled target. Microarrays are produced by spotting up to 10 000 polymerase chain reaction products, or more, representing specific genes, onto a glass or plastic slide matrix (Schena *et al.* 1995). Following purification and quality control, a few nanolitres of the polymerase chain reaction product are printed on coated glass microscope slides using a computer-controlled high-speed robot. Total RNA from both the test and reference samples is labelled with fluorescent tags (for example, red and green dye) by a single round of reverse transcription. The matrix is then simultaneously hybridized with the two resulting fluorescently-labelled cDNA in a competitive manner, and fluorescence scanners are used for detection (Schena *et al.* 1995, 1996; Fig. 1). The high-density oligonucleotide arrays differ from the microarrays in that the DNA probe is generated *in situ* on the surface of the matrix by a method called photolithography. These arrays (Genechips<sup>®</sup>; Affymetrix, Santa Clara, CA, USA) can contain between 40 000 and 60 000 probes, providing the highest density of probes of any array. However, the probes are limited in length and therefore specificity, and a mismatch detection scheme has to be used to determine specific hybridization (Lipshutz *et al.* 1999; Freeman *et al.* 2000). The microelectronic arrays, the newest hybridization array, consist of sets of electrodes covered by a thin layer of agarose coupled with an affinity moiety which provides controlled electrophoretic fields (Cheng *et al.* 1998; Heller *et al.* 2000).

Analysis and handling of the array data is one of the most difficult aspects in the utilization of the technology. The images of the scanner are imported into software which has to transform the fluorescence data into information about the clones, such as gene name, clone identifier, intensity values, intensity ratios, normalization constant and CI (Duggan *et al.* 1999). Clustering algorithms (hierarchical and non-hierarchical) have been the most used tools for analysing array data, and make it possible to find groups of genes or clusters with similar behaviour (Brazma & Vilo, 2000; Celis *et al.* 2000).

Due to the adaptable nature of the fabrication and hybridization methods, these techniques have been widely applied. Hybridization arrays have been applied to the discovery of sets of genes that have roles in diseases such as cancer (DeRisi *et al.* 1996; Kononen *et al.* 1998; Cole *et al.* 1999; Marx, 2000; Sallinen *et al.* 2000), insulin resistance (Aitman *et al.* 1999), hypertension (Lee *et al.* 2000), rheumatoid arthritis and inflammatory bowel disease (Heller *et al.* 1997).



**Fig. 1.** cDNA microarray schema: polymerase chain reaction (PCR) products representing specific genes are robotically printed on a glass or plastic matrix. RNA samples from lean and obese individuals are fluorescently labelled by a single round of reverse transcription, and then competitively hybridized to the same array. Fluorescent scanners are used for detection and the microarray data are analysed with sophisticated computer programs.

cDNA biochips, therefore, constitute an extraordinary tool for use by nutritionists (Trayhurn, 1998) and obesity researchers to identify those combinations of genes (known and unknown) that are implicated in the development of obesity and other nutrition-related disorders. A recent study has reported on the use of DNA microarray technology to assess the changes in gene expression associated with obesity and diabetes in lean, obese and diabetic mice. The expression level of >11 000 transcripts was analysed, finding that the expression of adipogenic genes was reduced in obesity and diabetes (Nadler *et al.* 2000). Oligonucleotide microarrays have also been used to study the components of the transcriptional programme specifically affected by leptin in *ob/ob* mice. This study found that many adipose tissue genes were differentially expressed between *ob/ob* and lean mice, and also identified a series of gene clusters that are specifically regulated by leptin and that distinguish leptin treatment from food restriction (Soukas *et al.* 2000). Microarray technology, therefore, is providing insight into the molecular events implicated in the development of obesity, diabetes and other nutritionally-related disorders that would be difficult to obtain using the traditional gene-by-gene approach.

Oligonucleotide microarray (DNA chip)-based hybridization analysis also constitutes a promising new technology for exploring the genome (Brown & Botstein, 1999). Thus, Gabrielsson *et al.* (2000) have performed a partial genome scale analysis of gene expression in human adipose tissue using DNA array. This study allowed the identification of numerous genes not previously reported to be expressed in adipose tissue, suggesting novel candidate genes involved in the pathophysiology of obesity (Gabrielsson *et al.* 2000).

Array technology can also be used for the screening of possible mutations and sequence variations in genomic

DNA (Hacia, 1999). Microarray technology, therefore, constitutes a useful tool in clinical diagnosis by detecting gene expression mutations associated with disease states in either biopsies or peripheral blood cells (Brugarolas *et al.* 2001). In this sense, a Gene Chip p53 assay has been used to detect the accumulation of p53 mutations during carcinogenesis and the development of gastrointestinal cancers (Takahashi *et al.* 2000). With regard to obesity, spontaneous mutations in five different genes have been found to be responsible for obesity in mouse models of obesity, i.e. the *obese (ob)*, *diabetes (db)*, the *agouti yellow (A<sup>y</sup>)*, the *fat* and *tubby* mutations. Mutations in some of these genes have also been related to the susceptibility to develop obesity in man (for review, see Bouchard *et al.* 1999). In addition, studies published in the last few years have provided evidence for an association between obesity-related phenotypes and polymorphisms in uncoupling proteins 2 and 3, peroxisome proliferator-activated receptor  $\gamma$ ,  $\beta_2$ -adrenergic receptor, apolipoprotein A4, insulin receptor substrate I (for review, see Chagnon *et al.* 2000). Furthermore, several groups reported additive effects of polymorphisms on the development of obesity. One example is the finding that the polymorphism in the uncoupling protein 1 and  $\beta_3$ -adrenergic receptor genes had an additive action on weight loss (Fogelholm *et al.* 1998; Kogure *et al.* 1998).

DNA hybridization arrays, therefore, represent a huge opportunity to study the association of a number of different polymorphisms with the development of obesity. In addition, the design of cDNA chips that allow the identification of possible mutations in candidate genes related to obesity might soon be very useful in clinical practice. The cDNA chips will help to characterize the aetiology of obesity and, therefore, a better approach for its

treatment. In addition, the early identification of the genetic predisposition to obesity will enable physicians to choose the appropriate preventive intervention for each patient, involving nutritional or exercise programmes. In this sense it has been shown that the association of some gene polymorphisms with an obese phenotype depends on the environment (diet and physical activity). For example, the Gln27Glu polymorphism of the  $\beta_2$ -adrenergic receptor gene is associated with high body weight and BMI in sedentary men but not in physically active men (Meirhaeghe *et al.* 1999).

DNA chips will also be very useful to study the effects of the type of nutrients in the diet on the expression of obesity-related genes. A recent study has already used DNA expression arrays to determine the effect of energy restriction on the age-related changes in gene expression (Han *et al.* 2000). It has been proposed that DNA chips will soon be used to monitor gene expression in response to a wide variety of nutritional events (Roberts *et al.* 2000).

DNA microarray technology is also being used in drug discovery and development. The opportunity to compare the expression of thousands of genes between 'disease' and 'normal' tissues and cells will allow the identification of multiple potential targets for therapeutic intervention (Debouck & Goodfellow, 1999). Two recent studies have used microarrays as a tool for investigating the mechanism of drug action (Gray *et al.* 1998; Marton *et al.* 1998). Differential gene expression technologies have also been used for elucidating both drug efficacy and toxicity, as well as novel candidate genes for pharmacogenetic analyses to assess individual variability to drug response (Nuwaysir *et al.* 1999; Rininger *et al.* 2000).  $\beta_3$ -Adrenergic agonists have been described as potential anti-diabetic and anti-obesity drugs, through effects on white adipose tissue. cDNA microarrays have been used to explore the molecular mechanisms of this response to  $\beta_3$ -adrenergic agonists. These studies found that the drugs alter the expression of some adipose tissue genes such as fibroblast growth factor and tumour necrosis factor  $\alpha$ -induced protein, which may modulate the  $\beta_3$ -adrenergic inhibition of food intake (Ye *et al.* 2000). The cDNA chips, therefore, can be useful tools for the discovery of anti-obesity drugs, as well as for the identification of their mechanisms of action.

In summary, cDNA array technology, despite some limitations such as the high cost, provides a powerful opportunity for obesity researchers and nutritionists. This technology constitutes the appropriate tool for studying those combinations of genes and mutations that are involved in the development of obesity in man, as well as for establishing the participation of environmental factors on the onset of obesity in individuals with a specific genetic background.

M. J. Moreno-Aliaga<sup>1</sup>, A. Marti<sup>1</sup>, J. García-Foncillas<sup>2</sup>  
and J. Alfredo Martínez<sup>1</sup>

<sup>1</sup>Department of Physiology and Nutrition and

<sup>2</sup>Cellular Therapy Unit  
University of Navarra  
31080 Pamplona  
Spain

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