

Horizons in Nutritional Science

Nutritional genomics: food industry applications from farm to fork

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Nutritional genomics is a new and promising science area which can broadly be defined as the application of high throughput genomics (transcriptomics, proteomics, metabolomics/metabonomics) and functional genomic technologies to the study of nutritional sciences and food technology. First utilised in the food industry by plant biotechnologists to manipulate plant biosynthetic pathways, the use of genomic technologies has now spread within the agriculture sector, unleashing a host of new applications (e.g. approaches for producing novel, non-transgenic plant varieties; identification of genetic markers to guide plant and animal breeding programmes; exploration of diet–gene interactions for enhancing product quality and plant/animal health). Beyond agriculture, genomic technologies are also contributing to the improvement of food processing, food safety and quality assurance as well as the development of functional food products and the evolution of new health management concepts such as ‘personalised nutrition’, an emerging paradigm in which the diet of an individual is customised, based on their own genomic information, to optimise health and prevent disease. In this review the relevance of nutritional genomics to the food industry will be considered and examples given on how this science area is starting to be leveraged for economic benefits and to improve human nutrition and health.

Nutrigenomics: Nutrigenetics: Gene–diet interactions: Functional food: Personalised nutrition

Recent technology developments have fundamentally changed the way in which biology is studied. In 2001 the first draft of the human genome sequence was published. Since then significant inroads have been made towards characterising biological processes at the molecular level, defining which genes are induced or repressed (the transcriptome), what proteins are produced (the proteome) and how these proteins influence metabolic processes (the metabolome). New insights have also been revealed about the way in which the environment can modify these molecular events and the way in which genotype may predict phenotype and influence responsiveness to environmental factors.

Nutritional genomics is a promising new research area and as a young science the way in which it is defined is still a topic for debate. For many researchers nutritional genomics is closely associated with ‘personalised nutrition’, an emerging concept in which the diet of an individual is customised, based on their own genomic/genetic information, to optimise health and prevent the onset of disease. In this context nutritional genomics is largely concerned with elucidating the interactive nature of genomic, dietary and environmental factors and how these interactions impact on health outcomes. One aim is to understand how diets and dietary components affect gene expression and how these changes in turn influence protein

expression and modification, metabolism and ultimately health (nutrigenomics). A second aim is to understand how genetic variation modifies an individual’s physiological response to diet and how this may influence parameters of health or disease (nutrigenetics). Alternatively the topic of nutritional genomics may be considered more generally, a broader definition being the application of high throughput genomics (transcriptomics, proteomics, metabolomics/metabonomics) and functional genomic technologies to the study of nutritional sciences and food technology (van der Werf *et al.* 2001). If viewed from this wider perspective then the impact of nutritional genomics on the food industry is considerable, with applications from ‘farm to fork’ anticipated (see Fig. 1). Indeed, in a number of instances these applications are no longer just a vision but are now approaching reality. In this review the relevance of nutritional genomics to the food industry will be considered and examples given on how this science area is starting to be leveraged for economic benefits and to improve human nutrition and health.

Nutritional genomics and agriculture

Plant biotechnologists were the first group within the food industry to seriously adopt and implement genomic science.

Abbreviations: CLPG, callipyge; DGAT1, diacylglycerol O-acyltransferase 1; FASN, fatty acid synthase; IGF2, insulin-like growth factor 2; MSTN, myostatin; MTHFR, methylenetetrahydrofolate reductase; PRKAG3, protein kinase; RYR1, ryanodine receptor1.

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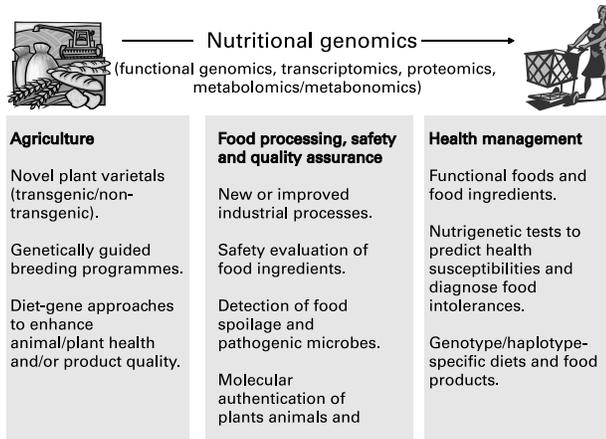


Fig. 1. Examples of how nutritional genomics is starting to be leveraged by the food industry for economic benefits and to improve human nutrition and health. Nutritional genomics can be defined as the application of genomics (functional genomics, transcriptomics, proteomics, metabolomics/metabonomics) to the study of nutritional science and food technology. If viewed in this broad manner then it is anticipated that multiple and varied applications, along the entire length of the food chain, will emerge from this science area.

Indeed, in 1999 the term ‘nutritional genomics’ was used to describe the successful manipulation of plant biosynthetic pathways to improve human health (DellaPenna, 1999). Agricultural interest in genomics has now expanded to the livestock industry. Although transgenic approaches to altering the genetic makeup of meat-producing animals are unlikely to be acceptable to many consumers, opportunities do exist for agricultural researchers to use marker-assisted selection to exploit natural variation. In a broad sense agriculture-based nutritional genomics is concerned with defining how to grow and feed domesticated crops and livestock to maximise their own health, resilience and yield as well as modifying their composition to improve nutritional qualities that are important for human health. Applications for nutritional genomics therefore include the identification of dietary signals that boost immunity, eliminating the need for antibiotic use in animal feed, as well as the development of crops or animal produce with increased levels of healthful phytochemicals.

Domesticated food crops

The combination of genomics and molecular biology has created a new way for scientists to generate plant varieties, one that offers wider functional scope and greater precision than conventional plant breeding methods. Although in the first instance GM plants were produced for pure agro-economic reasons, to achieve resistance to herbicides or to pathogens (mainly insects or viruses), more recently the technology has been focused towards improving the nutritional qualities of plants and enhancing human health.

A good example of the nutritional benefits that can be achieved through genetic modification is Golden Rice, a rice strain engineered to produce β -carotene (pro-vitamin A) in the endosperm and designed as a biotechnology solution to the problem of vitamin A deficiency. Vitamin A deficiency is a significant public health problem in many parts of the developing world, particularly Africa and South East Asia

where rice is a primary food staple. It is the leading cause of preventable blindness in children and may increase the incidence and severity of infectious diseases. In a proof of concept study, scientists showed that it was possible to establish an entire biosynthetic pathway *de novo* in rice endosperm, enabling the accumulation of pro-vitamin A (Ye *et al.* 2000). In the original strains pro-vitamin accumulation was relatively limited, supplying only 15–20% of the recommended dietary allowance for vitamin A. However, recently Golden Rice 2 has appeared; this new variety accumulates pro-vitamin A at levels more than 20-fold higher than those of the original and could deliver up to 50% of a child’s vitamin A requirements (Paine *et al.* 2005).

A further example of using genetic modification to improve nutritional traits is the development of transgenic tomatoes. In an example of such work a gene from *Petunia* encoding chalcone isomerase, an important flavonoid biosynthesis enzyme, was over-expressed in tomatoes. Processing of the resultant high-flavonol tomato fruit generated a tomato paste containing levels of flavonols that were 21-fold higher than the paste manufactured from a standard fruit (Muir *et al.* 2001). Flavonols are known to exhibit antioxidant properties and consumption of flavonol-rich foods has been linked to improvements in health, particularly cardiovascular health (Fisher & Hollenberg, 2005).

Despite the nutritional benefits offered by GM food and crops the public reaction to this technology has been poor, particularly in Europe. Indeed, the progress of GM crops through the European Union regulatory system largely halted in 1998 pending a review of all regulations pertaining to the release of GM organisms and the marketing of GM products (Hails & Kinderlerer, 2003). Given this, some plant biotechnologists have sought alternative ways of using genomic information to deliver crop improvements. This has led to the development of Targeting Induced Local Lesions IN Genomes (TILLING), a technique that shows considerable promise as a non-transgenic way of improving domesticated crops (Slade & Knauf, 2005). Essentially TILLING is a reverse genetics technique. Rather than engineering a new cultivar with specific characteristics, technologists identify variants with the desired genetic characteristics by high throughput screening of large natural germplasm collections or novel, chemically mutagenised populations. Although originally developed for use in *Arabidopsis thaliana*, a diploid model organism, TILLING has also been successfully applied to a range of other plant species (soyabean, maize, romaine and iceberg lettuce, rice, peanut, castor) including those with more complex genomes, such as durum (tetraploid) and bread (hexaploid) wheat (Slade & Knauf, 2005).

Livestock and animal produce

Selective breeding has been applied for thousands of years to improve desirable characteristics (e.g. disease resistance) in domesticated animal species. With the advent of genomic technologies efforts are now underway to understand the genetic basis of commercially important traits. In animals quantitative trait loci, multiple genomic regions identified as important in determining the variation of a complex phenotype, have been identified for a range of economically important traits including, growth, fatness, fertility, milk production/

composition, meat quality and health (Harlizius *et al.* 2004; Gordon *et al.* 2005). The molecular basis of these phenotypes is complex and for the most part remains uncertain, with only a small number of genes in quantitative trait loci identified to date. However, as knowledge builds and the genetic variations underlying these traits are more comprehensively delineated opportunities may emerge for stock improvement through marker assisted selection. Indeed, genotyping services (www.cogenics.com) to test for markers associated with scrapie susceptibility and meat tenderness are already being offered to the agricultural community.

The past decade has also seen a concerted effort to map the genomes of all major farm animal species. Initially efforts were focused on pigs, cattle and chicken. However, medium-density genetic linkage maps are now also available for horses, goats and several fish species (Harlizius *et al.* 2004). More recently, the sequencing of farm animal genomes has begun. The first draft of the chicken (*Gallus gallus*) genome was published in 2004 and a genetic variation map, based on a comparison of the sequence of three domestic chicken breeds (a broiler, a layer and a chinese silkie) with that of the red jungle fowl, the predecessor of the domestic chicken, has also been developed (Hillier *et al.* 2004; Wong *et al.* 2004). Despite the lack of detailed sequence maps new insights on how genetics influence commercially important traits have started to emerge. For example, the genes *PPARGCIA*, *DGAT1* and *FASN* have been shown to influence the fat content of milk (Pareek *et al.* 2005; Weikard *et al.* 2005; Roy *et al.* 2006), *RYR1*, *MSTN*, *PRKAG3*, *IGF2* and *CLPG* are all associated with muscle growth, the major determinant of the performance of meat-producing animals (Gordon *et al.* 2005) and polymorphisms in the prolactin and prolactin receptor genes have been linked to broodiness in chickens (Jiang *et al.* 2005). However, for some of these genes, particularly those linked to musculature, negative side effects have also been observed. In pigs the high muscle growth associated with the *RYR1* and *PRKAG3* genes has been linked to reduced meat quality and the *MSTN* gene is associated with a reduction in stress tolerance, calf viability and female fertility (Harlizius *et al.* 2004; Gordon *et al.* 2005).

In addition to studying direct gene effects, agricultural researchers have also started to explore the way in which genetic factors may interact with diet. Although this field of research is still very much in its infancy there is considerable excitement about what the future may hold, particularly the impact such interactions may have on the nutritional value of food products (meat, butter, milk, cheese and eggs, etc.). For example, supplementation of dairy cattle diets with plant oils high in linoleic and/or linolenic acid is known to increase milk levels of *cis*-9, *trans*-11 conjugated linoleic acid, a putative anti-cancer agent. Furthermore, striking differences between individual animals in the degree of conjugated linoleic acid enrichment have been observed and may, in part, be the result of genetic factors (Lock & Bauman, 2004). A second example is eggs and milk containing increased amounts of *n*-3 fatty acids compared to conventional products. Diets rich in *n*-3 fatty acids, such as DHA and EPA, have long been considered as beneficial to human health, particularly against CVD (Bautista & Engler, 2005). However, the *n*-3 content of western diets tends to be relatively low. As a consequence, opportunities to enhance *n*-3 fatty acids in many

foods are being explored. Supplementation of cattle feedstuffs with fish oils, fish by-products and marine algae is known to increase DHA and EPA levels in milk (Lock & Bauman, 2004), whilst poultry fed flaxseed produce eggs containing high levels of α -linolenic acid, a plant-derived *n*-3 fatty acid (Bourre, 2005). Whether this enrichment can be improved through genotype selection is still to be determined. However, genetic strain has been shown to influence the incorporation of *n*-3 fatty acids into the yolk of chicken eggs suggesting that it may be possible (Steinhilber, 2005).

Nutritional genomics and food processing, food safety and quality assurance

Moving beyond agriculture to other parts of the food chain, researchers are now using genomic technologies to drive improvements in food processing, food safety and quality assurance. Applications include the use of 'DNA finger printing' to check the authenticity of food ingredients and to verify the composition of processed food products as well as the development of molecular-based diagnostics to guide food processes, predict the shelf-life of fresh produce and detect microbiological contamination.

Food processing

A relatively new application of genomic technologies is the discovery of 'process markers', informative molecular markers that may be used to guide industrial processes or improve supply chain management. For example, the manufacture of tea is a complex process, involving multiple steps (withering, 'fermentation', heat processing and drying) that can influence both the aroma and taste of the final product. Defining the molecular basis of these processes should help improve current manufacturing techniques and may also highlight alternative, perhaps more scaleable, manufacturing approaches. In terms of supply chain management molecular markers may prove useful for predicting and improving the shelf-life of fresh produce. Broccoli, a popular green vegetable, has a notoriously short shelf-life, yellowing and losing turgor within days of harvest. Researchers studying the way in which genetic and environmental factors influence post-harvest performance have revealed that the processes occurring in harvested broccoli are highly comparable to those that occur during normal leaf senescence (Page *et al.* 2001). Genomic approaches have been used to identify and characterise a range of senescence-enhanced genes in the model plant species *Arabidopsis*, allowing a putative network of signalling pathways to be proposed (Buchanan-Wollaston *et al.* 2003). As understanding of the molecular basis of plant senescence continues to develop it should enable the rational design of growth and harvesting techniques to improve post-harvest crop performance. Indeed, knowledge gained from leaf senescence studies has already been used to manipulate the post-harvest characteristics of broccoli and lettuce (Henzi *et al.* 2000; Chen *et al.* 2001; McCabe *et al.* 2001).

Food safety

To date, the use of genomics in food safety has concentrated on two main areas, the safety evaluation of food components

(van Ommen & Groten, 2004) and the detection of micro-organisms which may cause food spoilage or be hazardous to human health (Abee *et al.* 2004). Safety evaluation of food components is concerned with both hazard identification (whether a food component causes an adverse health effect), and hazard characterisation (the level of exposure required to elicit an adverse health effect). This data is then used to determine the acceptable daily intake of a particular food or food chemical. Although hazard analysis is clearly important, gathering appropriate data can be costly and time consuming, requiring detailed toxicological experimentation in animals, often on an empirical basis.

Genomic technologies can offer a number of benefits when conducting toxicological evaluation. Firstly, their high throughput nature means that it is possible to analyse multiple tissues in a timely and cost-effective manner. Secondly, by applying transcriptomics, proteomics and metabolomics/metabonomics the full range of biological responses from gene expression through to cellular functions can be studied. Thirdly, and perhaps most importantly, mechanistic understanding is not a prerequisite for good experimental design. In fact, genomic technologies may usefully be applied as hypothesis generation as well as hypothesis testing tools. As knowledge grows on the gene, protein and metabolic changes induced by particular xenobiotics it should be possible to derive mechanistic insights for new compounds by comparing their profiles with existing data. Furthermore, such comparisons may prove useful in highlighting potential toxicities early on, enabling more targeted toxicological analysis. The utility and challenges of using genomic technologies in toxicological assessment has recently been reviewed by a number of authors (Battershill, 2005; Heijne *et al.* 2005; Lindon *et al.* 2005; Reynolds, 2005).

A second application of genomics to food safety is the control and detection of food borne micro-organisms. Traditional means of controlling microbial spoilage and safety hazards in foods include freezing, blanching, sterilisation, curing and use of preservatives. However, the developing consumer trend for 'naturalness', as indicated by the strong growth in sales of organic and chilled food products, has resulted in a move towards milder food preservation techniques. This raises new challenges for the food industry.

The first bacterial genome sequence was completed over a decade ago. Now genome sequences are available for many of the microbes that cause food borne diseases, including *Listeria monocytogenes*, *Yersinia enterocolitica*, *Escherichia coli*, *Clostridium botulinum* A, *Campylobacter jejuni* and various *Salmonella* species (Abee *et al.* 2004). This information combined with comparative genomic techniques may help microbiologists to identify unique, strain-specific genetic signatures which can be used for detecting and typing microbial contamination. For example, comparison of the genome data of ninety-seven strains of *C. jejuni* has recently been reported (Taboada *et al.* 2004). Gene divergence in *C. jejuni* is largely restricted to a small number of genomic 'hotspots'. Many genes located at these variability loci are divergent across multiple strains. However, a large number are also unique to a single strain. Genes that display a high degree of intra-species variability represent good targets for genotype detection purposes. Genetic-based detection of microbes offers significant benefits over conventional detection methodology,

particularly from a human health perspective. Firstly, multiple species and strains, including virulence markers, can be analysed simultaneously. Secondly, a much more rapid turn around of test results can be achieved. Indeed, for current PCR-based methods results can be produced in 24–48 h, whilst advances in direct DNA detection techniques, i.e. methods that do not require target amplification by PCR, should facilitate the development of robust, portable systems, reducing analysis time from hours to minutes as well as enabling 'in the field' testing. For example, one such system, which is currently under development at Integrated-Nanotechnologies (www.integratednano.com), can directly detect the binding of a target DNA molecule to sensors on a microchip surface. Detection involves metallisation of the bound target, creating a highly conductive DNA wire which links the previously isolated sensors.

Genomic technologies can also help scientists to derive better understanding of the life cycles of bacteria. Defining the mode of action of food borne bacteria and the mechanisms that confer 'stress resistance' should enable more rational design of food preservation techniques. In addition, this information can also be used to pinpoint areas of the food chain that are most susceptible to microbial contamination.

Quality assurance

DNA identification is now being applied within the food industry as a means of authenticating plants, animals and packaged food products.

One application of genetic analysis is as a means of authenticating and controlling conventional animal identification systems. National disease monitoring and eradication programmes depend heavily on conventional animal identification, usually with ear tags. Protecting the integrity of these programmes is vital to their success. However, conventional methods are open to fraudulent practices such as tag swapping and may be further compromised by animal theft and smuggling. In contrast, DNA is largely unalterable and is an integral part of the animal. These properties not only enable the verification of live animals but also allow complete traceability of foodstuffs throughout the length of the food chain.

A second application is the molecular authentication of food ingredients and packaged food products. Consumers rely heavily on food labelling to guide product choice, particularly if the food has been processed, removing the ability to distinguish one ingredient from another. As food choice often reflects important personal beliefs, as well as health concerns, it is essential that food labelling is honest and accurate. Authentication of foodstuffs may be carried out by manufacturers as part of their quality assurance processes, either to check the provenance of supplied ingredients or to detect for cross-contamination during production. It may also be used by food standard agencies as a means of detecting 'food fraud' and prosecuting fraudsters. Regional and traditional ingredients that have particular consumer appeal can attract a premium price. For example, traditional Basmati rice, which is known for its superior aroma and grain quality, commands a higher market price than cross-bred Basmati and non-Basmati rice. Consequently certain food stuffs may be prey to illegal practices, including adulteration or even

complete substitution of the premium ingredient with a less costly alternative. Indeed, substitution of tuna with inferior 'bonito' fish species has been reported in the canned fish industry and adulteration of hard wheat (*Triticum durum*) derived flour with cheaper common wheat (*Triticum aestivum*) flour is known to be a problem in the pasta industry (Woolfe & Primrose, 2004).

Checking the legitimacy of ingredients can be difficult for food manufacturers, particularly when key appearance characteristics, such as head, skin and fins in the case of fish, are removed. However, genetic analysis appears to offer a promising solution. Not only is DNA a highly robust molecule, capable of withstanding the rigours of food processing (e.g. sterilisation at temperatures of > 100°C), it is also species unique and present in all parts of the plant or animal. In the UK, the government funded Central Services Laboratory (www.csl.gov.uk) has recently developed a microsatellite-based technique to distinguish between different varieties of Basmati and long grain rice (Woolfe & Primrose, 2004). By analysing a range of repetitive elements the test is able to identify the presence of just 5% non-Basmati grain in a sample. Other researchers have also identified genetic markers that can distinguish traditional, cross-bred and non-Basmati rice varieties (Jain *et al.* 2004). Further examples of this type of application include genetic tests, developed in Japan, that can distinguish premium Koshihikari rice from cheaper varieties and genuine Tochiotome strawberries from inappropriately labelled Korean fruit (Williams, 2005). In addition, a high density DNA chip, FoodExpert-ID[®] (bio-Mérieux, Marcy L'Etoile, France), for detecting multiple animal species in either packaged food products or animal feed has been developed in Europe.

Nutritional genomics and human health

For thousands of years it has been recognised that nutrition plays a crucial role in the development of disease. Conversely, diet can play an equally important role in disease management. A classic example is scurvy, a devastating health condition that is caused by vitamin C deficiency but which is readily addressable, even in individuals with advanced disease, with vitamin C supplementation. Many disorders caused by vitamin and mineral deficiency have largely been eradicated in the developed world through fortification of common food stuffs. For example, salt has been fortified with iodine to prevent goitre and vitamins A and D added to milk to prevent rickets. In response to this success attention has now turned to the role played by diet and lifestyle in the development and management of age-related chronic diseases, particularly diabetes, CVD and cancer.

Globally the prevalence of diabetes is increasing, with conservative estimates predicting that the worldwide incidence will rise above 350 million by 2030 (Wild *et al.* 2004). This rising disease burden is due primarily to population growth, aging, urbanisation and the increasing prevalence of obesity. Recent studies have shown how powerful diet and lifestyle modification can be for preventing the development of type 2 diabetes, particularly in high risk individuals with impaired glucose tolerance (Tuomilehto *et al.* 2001; Knowler *et al.* 2002; Li *et al.* 2002). Indeed, in the NIH-sponsored Diabetes Prevention Programme not only was diet and lifestyle

modification shown to be a successful strategy it was also shown to be more effective than pharmacological treatment and estimated as being economically more attractive (Herman *et al.* 2005). Findings from the various diabetes prevention trials have helped to heighten awareness of the connection between nutrition and disease. Furthermore, they have stimulated considerable interest in functional foods and nutrition-based health management, two areas in which genomic technologies are anticipated to have substantial impact.

Functional foods

The daily consumption of margarine fortified with plant sterols has been shown to significantly reduce serum cholesterol levels compared with consumption of a comparable unfortified spread (Hendriks *et al.* 1999). Cholesterol-lowering spreads fall into a relatively new product category known as functional foods. These are food products that have, or claim to have, a specific health-promoting or enhancing effect over and above their nutritional content. Products may be focused on disease prevention (e.g. phytosterol-containing products for cholesterol-lowering) or enhancing daily health and wellbeing (e.g. pro-biotic products for digestive health).

Understanding how foods and food components modulate health is a core technology requirement for the development of functional foods. Epidemiological studies have repeatedly shown associations between food intake and the incidence and severity of disease. However, identifying the bioactive components of foodstuffs and defining their mode of action is challenging. Not only are diets highly complex, consisting of many separate food items, but each food item itself is a complex mix of bioactive components. To help understand this complexity nutritional scientists are starting to turn to the genomic tool box. For example, high density DNA and protein arrays can be used to delineate changes in gene and protein expression induced by whole diets, dietary constituents or individual phytochemicals. Comparison of the molecular changes post-intervention may directly help to identify bioactive components. However, more importantly, they can help nutritional researchers to elucidate diet-responsive genes and pathways, providing molecular targets for the development of *in vitro* screens, which in turn can be used to define the bioactive components of diets and individual food stuffs. To date, transcriptomics has been more readily embraced by the nutrition research community than proteomics. However, as new and improved proteomic techniques emerge this will undoubtedly change. The ability to integrate gene and protein expression data as well as to map post-translational modifications and even amino acid substitutions to particular cellular functions, offers a powerful approach for understanding the molecular basis of nutrition. Indeed, the application of proteomic analysis to cell and animal models, clearly illustrates the potential of this technique (Fuchs *et al.* 2005).

A second important aspect for functional food development is the availability of appropriate biomarkers for evaluating efficacy. This is particularly important if the goal is to prevent rather than treat health conditions. Indeed, in the case of plant stanol/sterol-based functional foods the ultimate goal is to reduce levels of morbidity and mortality by improving cardiovascular health. However, the focus of efficacy studies is measurement of serum levels of LDL cholesterol, an accepted

CVD risk factor. Genomic technologies can be used to characterise the development of disease states to identify novel biomarkers. Alternatively they can be used to map the molecular and physiological response to interventions in order to identify efficacy markers. In particular, metabolomics and metabonomics, emerging disciplines that aim to globally measure low-molecular-weight molecules in biological systems, are well placed to impact on biomarker discovery and to explore the complex relationship between nutrition, metabolism and disease (Whitfield *et al.* 2004). For example, $^1\text{H-NMR}$ -based metabolomics has been successfully applied to the diagnosis of coronary artery disease, analysis of the spectral data providing information on both the presence and severity of disease (Brindle *et al.* 2002) and has also been used to explore the relationship between serum metabolite profiles and hypertension (Brindle *et al.* 2003). More recently, researchers have started to apply metabonomics to human nutritional research, examples to date include the use of $^1\text{H-NMR}$ -based metabolomics to characterise the metabolite changes in plasma and urine in response to an isoflavone dietary intervention (Solanky *et al.* 2003, 2005) and characterisation of the changes in urinary metabolites in response to daily chamomile tea ingestion (Wang *et al.* 2005).

Nutrigenetic testing and personalised nutrition

Recent advances in genomic and informatic technologies have resulted in a shift away from traditional epidemiology (understanding how diet, lifestyle and societal factors influence the causes, distribution and control of disease) and a move towards molecular epidemiology (understanding the interactive nature of gene, diet and environmental factors and how these interactions may impact on health outcomes). Although 99.9% of human DNA sequences are identical, the 0.1% difference between any two individuals has profound biological significance. Not only do people look different, but they also react differently. For example, work conducted by Schaefer and colleagues showed that there is considerable variability in plasma lipid response to a standard cholesterol-lowering diet (Schaefer *et al.* 1997), observed changes in LDL cholesterol level ranging from +3% to -55% in men and from +13% to -39% in women. This differential response is, in part, the result of genetic variation.

Understanding of the full complexity of genetic factors that underpin such observations is currently limited. However, a number of examples of single gene-diet interactions have been reported, resulting in the development of nutrigenetic testing as a new paradigm for health management. Nutrigenetic testing focuses on testing for DNA polymorphisms which are known to influence phenotypic responses to diet. The purpose of testing is to use this genotype information to provide more personalised advice about nutrition and health, the primary goal being to use an individual's genetic information to predict their future health susceptibilities and to guide selection of 'the best' preventative action. A well studied example of a diet-gene interaction which illustrates this concept is the interaction between the *MTHFR* gene, dietary folate and plasma homocysteine levels. A C/T change at position 677 in the *MTHFR* gene results in production of a partially defective (about 35% of normal enzyme activity) form of methylenetetrahydrofolate reductase (*MTHFR*) an

enzyme which is involved in regulating folate metabolism and lowering plasma levels of the amino acid homocysteine. Consistent with this, the TT genotype is associated with elevated plasma levels of homocysteine when folate status is low (Saw *et al.* 2001), implying that individuals who are *MTHFR* positive (i.e. possess the *MTHFR* TT genotype) may require higher levels of dietary folate to prevent the development of hyperhomocysteinemia. Indeed, dietary intervention with folic acid is known to reduce homocysteine levels and individuals with the *MTHFR* TT genotype appear to be particularly responsive to the intervention (Miyaki *et al.* 2005). Findings from observational studies have implicated hyperhomocysteinemia as a modifiable risk factor for CHD, stroke and dementia and indicate that individuals carrying the *MTHFR* TT genotype have an elevated risk of developing CVD (Strain *et al.* 2004; Clarke, 2005; Troen & Rosenberg, 2006). As such, nutrigenetic testing for the *MTHFR* C677T polymorphism would seem to offer a route for identifying individuals with particular health susceptibilities that can be addressed through dietary means. In contradiction to this, recent findings from a number of randomised, controlled trials suggest that homocysteine lowering, through dietary supplementation with B vitamins, does not reduce CVD risk or improve cognitive performance (Stott *et al.* 2005; Bonaa *et al.* 2006; Lonn *et al.* 2006; McMahan *et al.* 2006). This has raised questions over the causal role of homocysteine in disease pathogenesis and the suggestion that elevated plasma homocysteine levels may represent a risk marker of disease rather than a risk factor (Seshadri, 2006). A further concern is the usefulness of testing for a single genetic marker in the context of a condition, in this case CVD, which is known to be highly polygenic and to have multiple environmental risk factors. In a recent meta-analysis the difference in effect size between *MTHFR* genotype groups for coronary artery disease risk though significant was relatively small, reflecting the contribution of multiple genetic factors to the final phenotype (Lewis *et al.* 2005). Even if consideration is limited to the *MTHFR* gene, dietary folate and plasma homocysteine levels, gene-gene interactions may still modify the end phenotype. For example, a functional interaction between two *MTHFR* polymorphisms (C677T and the A1298C) has recently been reported (Ulvik *et al.* 2006). Thus genetic testing of single gene variants may provide additional information about disease aetiology and the role of diet. However, the predictive nature of such tests will be highly limited. Indeed, if nutrigenetic testing is to contribute significantly to improvements in health management then research needs to shift a way from single gene-diet interactions and focus on the interplay between multiple genetic and environmental factors.

An alternative and perhaps more attractive early application of nutrigenetic testing is the diagnosis of food intolerances, particularly monogenic conditions such as lactose intolerance. Intestinal lactase is essential for the digestion of lactose, a carbohydrate found in milk and other dairy products. In most mammals, including man, lactase activity declines after weaning. This maturational decline renders most of the world's adult population intolerant to lactose-containing food products. However, a minority of adults, primarily Northern Europeans or those with Northern European ancestry, display adult lactase persistence. Recently, genetic variation in the *Lactase* gene, a single nucleotide polymorphism (C/T) at

position – 13910, was shown to be strongly associated with lactase persistence/non-persistence (Enattah *et al.* 2002). The CC genotype completely associates with biochemically verified lactase non-persistence. Furthermore, molecular epidemiology studies have shown that the frequency of this genotype is in good agreement with the prevalence data for lactose intolerance in >70 ethnically diverse populations (Jarvela, 2005). Lactose intolerance can have a major impact on health and vitality. Primarily associated with gastric upset, lactose consumption by a lactose-intolerant individual, may also result in a range of systemic symptoms, including headache, severe tiredness, muscle and joint pain and allergy (Matthews *et al.* 2005). It has also been associated with a reduction in peak bone mass and increased susceptibility to osteoporosis (Sibley, 2004). Genetic-based diagnosis of lactose intolerance has significant advantages over the conventional lactose tolerance test. Firstly, it is less costly and labour intensive. Secondly, and more importantly it avoids the symptoms induced by lactose challenge, which can be debilitating for up to 3 d for some individuals (Sibley, 2004).

Although the science underpinning nutrigenetic testing is still immature the business outlook is viewed as encouraging. Several key driving forces including science, technology and consumer appeal are converging that should encourage growth of this area. Indeed, consumer research studies indicate that American consumers are already receptive to having their diet tailored to their genetic make up. In response to this emerging 'consumer pull' a number of early-stage companies (e.g. Sciona, Interleukin Genetics), focused on developing nutrigenetic tests for the consumer market, have started to appear. In addition, several food ingredient companies have begun investing in this area and a number of packaged food companies are actively engaged with European Union-funded research initiatives, including LIPGENE (Nugent, 2005) and DIOGenes (Saris & Harper, 2005). However, this burgeoning commercial excitement needs to be tempered by a certain degree of caution.

Even though evidence is rapidly accumulating to support the concept of personalised nutrition, reports on the clinical utility and validity of specific nutrigenetic markers are still rare. Studies investigating the sensitivity and specificity of markers linked to monogenic conditions, such as lactose intolerance have started to emerge and do show promising clinical value (Rasinpera *et al.* 2004; Hogenauer *et al.* 2005; Matthews *et al.* 2005). However, for complex polygenic traits, such as CVD or diabetes, generating such evidence is a much more challenging proposition. In the case of CVD not only is disease trajectory influenced by multiple genetic and lifestyle factors but it is also characterised by multiple biological risk factors (e.g. blood pressure, weight, serum cholesterol, LDL cholesterol, HDL cholesterol, TAG, C-reactive protein, etc.) and multiple disease outcome measures (e.g. all cause mortality, fatal CHD, non-fatal myocardial infarction, stroke, angina, coronary revascularisation, congestive heart failure, etc.). Disentangling this degree of complexity will not only take time but will also require significant research investment and the development of large interdisciplinary research consortia (Kaput *et al.* 2005). In addition to these technical issues consensus also needs to be reached on a large number of ethical and regulatory issues (Chadwick, 2004). Should

nutrigenetic testing be delivered directly to consumers or through healthcare professionals? Who should be tested? Who should have access to test information? How should individual privacy be protected? How should genetic discrimination be prevented? The way in which these and other issues are resolved will have a significant impact on the nutrigenetic business environment. Finally, consideration needs to be given to the way in which the consumer benefits of nutrigenetic science are communicated. The technology backlash experienced by plant biotechnologists over the introduction of GM foods and crops serves as an important lesson for the food industry, emphasising the need for early and proactive public engagement, and the development of communication strategies that clearly articulate the benefits, both for the individual consumer and the population at large, as well as the risks of nutrigenetic technology.

Conclusions

Nutrition plays a crucial role in health as well as disease. Despite its youth, nutritional genomics is already influencing multiple aspects of the food chain (agriculture, food production, food safety and quality assurance) and is starting to be leveraged more widely to deliver economic benefits and to enhance aspects of human nutrition and health. Over time nutritional genomics should accelerate the development of functional food products as well as extend knowledge and understanding of gene–diet interactions, a key building block for the future development of personalised nutrition.

References

- Abee T, van Schaik W & Siezen RJ (2004) Impact of genomics on microbial food safety. *Trends Biotechnol* **22**, 653–660.
- Battershill JM (2005) Toxicogenomics: regulatory perspective on current position. *Hum Experimental Toxicol* **24**, 35–40.
- Bautista MC & Engler MM (2005) The Mediterranean diet: is it cardioprotective? *Prog Cardiovasc Nurs* **20**, 70–76.
- Bonaa KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, Wang H, Nordrehaug JE, Arnesen E & Rasmussen K (2006) Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med* **354**, 1578–1588.
- Bourre JM (2005) Where to find omega-3 fatty acids and how feeding animals with diet enriched in omega-3 fatty acids to increase nutritional value of derived products for human: what is actually useful? *J Nutr Health Aging* **9**, 232–242.
- Brindle JT, Antti H, Holmes E, *et al.* (2002) Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabolomics. *Nat Med* **8**, 1439–1444.
- Brindle JT, Nicholson JK, Schofield PM, Grainger DJ & Holmes E (2003) Application of chemometrics to H-1 NMR spectroscopic data to investigate a relationship between human serum metabolic profiles and hypertension. *Analyst* **128**, 32–36.
- Buchanan-Wollaston V, Earl S, Harrison E, Mathas E, Navabpour S, Page T & Pink D (2003) The molecular analysis of leaf senescence – a genomics approach. *Plant Biotechnol J* **1**, 3–22.
- Chadwick R (2004) Nutrigenomics, individualism and public health. *Pro Nutr Soc* **63**, 161–166.
- Chen LFO, Hwang JY, Charng YY, Sun CW & Yang SF (2001) Transformation of broccoli (*Brassica oleracea* var. *italica*) with isopentenyltransferase gene via *Agrobacterium tumefaciens* for post-harvest yellowing retardation. *Mol Breeding* **7**, 243–257.

- Clarke R (2005) Homocysteine-lowering trials for prevention of heart disease and stroke. *Sem Vasc Med* **5**, 215–222.
- DellaPenna D (1999) Nutritional genomics: manipulating plant micronutrients to improve human health. *Science* **285**, 375–379.
- Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L & Jarvela I (2002) Identification of a variant associated with adult-type hypolactasia. *Nat Genet* **30**, 233–237.
- Fisher ND & Hollenberg NK (2005) Flavanols for cardiovascular health: the science behind the sweetness. *J Hypertens* **23**, 1453–1459.
- Fuchs D, Winkelmann I, Johnson IT, Mariman E, Wenzel U & Daniel H (2005) Proteomics in nutrition research: principles, technologies and applications. *Br J Nutr* **94**, 302–314.
- Gordon ES, Gordish Dressman HA & Hoffman EP (2005) The genetics of muscle atrophy and growth: The impact and implications of polymorphisms in animals and humans. *Int J Biochem Cell Biol* **37**, 2064–2074.
- Hails R & Kinderlerer J (2003) The GM public debate: context and communication strategies. *Nat Rev Genet* **4**, 819–825.
- Harlizius B, van Wijk R & Merks JW (2004) Genomics for food safety and sustainable animal production. *J Biotechnol* **113**, 33–42.
- Heijne WH, Kienhuis AS, van Ommen B, Stierum RH & Groten JP (2005) Systems toxicology: applications of toxicogenomics, transcriptomics, proteomics and metabolomics in toxicology. *Expert Rev Proteomics* **2**, 767–780.
- Hendriks HF, Weststrate JA, van Vliet T & Meijer GW (1999) Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects. *Eur J Clin Nutr* **53**, 319–327.
- Henzi MX, Christey MC & McNeil DL (2000) Morphological characterisation and agronomic evaluation of transgenic broccoli (*Brassica oleracea* L. var. *italica*) containing an antisense ACC oxidase gene. *Euphytica* **113**, 9–18.
- Herman WH, Hoerger TJ, Brandle M, Hicks K, Sorensen S, Zhang P, Hamman RF, Ackermann RT, Engelgau MM & Ratner RE (2005) The cost-effectiveness of lifestyle modification or metformin in preventing type 2 diabetes in adults with impaired glucose tolerance. *Ann Intern Med* **142**, 323–332.
- Hillier LW, Miller W, Birney E, *et al.* (2004) Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* **432**, 695–716.
- Hogenauer C, Hammer HF, Mellitzer K, Renner W, Krejs GJ & Toplak H (2005) Evaluation of a new DNA test compared with the lactose hydrogen breath test for the diagnosis of lactase non-persistence. *Eur J Gastroenterol Hepatol* **17**, 371–376.
- Jain S, Jain RK & McCouch SR (2004) Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labeled microsatellite markers. *Theor Appl Genet* **109**, 965–977.
- Jarvela IE (2005) Molecular genetics of adult-type hypolactasia. *Ann Med* **37**, 179–185.
- Jiang RS, Xu GY, Zhang XQ & Yang N (2005) Association of polymorphisms for prolactin and prolactin receptor genes with broody traits in chickens. *Poult Sci* **84**, 839–845.
- Kaput J, Ordovas JM, Ferguson L, *et al.* (2005) The case for strategic international alliances to harness nutritional genomics for public and personal health. *Br J Nutr* **94**, 623–632.
- Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA & Nathan DM (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* **346**, 393–403.
- Lewis SJ, Ebrahim S & Smith GD (2005) Meta-analysis of MTHFR 677C → T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? *Br Med J* **331**, 1053–1056A.
- Li G, Hu Y, Yang W, Jiang Y, Wang J, Xiao J, Hu Z, Pan X, Howard BV & Bennett PH (2002) Effects of insulin resistance and insulin secretion on the efficacy of interventions to retard development of type 2 diabetes mellitus: the DA Qing IGT and Diabetes Study. *Diabetes Res Clin Pract* **58**, 193–200.
- Lindon JC, Keun HC, Ebbs TMD, Pearce JMT, Holmes E & Nicholson JK (2005) The Consortium for Metabonomic Toxicology (COMET): aims, activities and achievements. *Pharmacogenomics* **6**, 691–699.
- Lock AL & Bauman DE (2004) Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids* **39**, 1197–1206.
- Lonn E, Yusuf S, Arnold MJ, *et al.* (2006) Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med* **354**, 1567–1577.
- McCabe MS, Garratt LC, Schepers F, Jordi WJRM, Stoopen GM, Davelaar E, van Rhijn JHA, Power JB & Davey MR (2001) Effects of P-SAG12-IPT gene expression on development and senescence in transgenic lettuce. *Plant Physiology* **127**, 505–516.
- McMahon JA, Green TJ, Skeaff CM, Knight RG, Mann JI & Williams SM (2006) A controlled trial of homocysteine lowering and cognitive performance. *N Engl J Med* **354**, 2764–2772.
- Matthews SB, Waud JP, Roberts AG & Campbell AK (2005) Systemic lactose intolerance: a new perspective on an old problem. *Postgrad Med J* **81**, 167–173.
- Miyaki K, Murata M, Kikuchi H, Takei I, Nakayama T, Watanabe K & Omae K (2005) Assessment of tailor-made prevention of atherosclerosis with folic acid supplementation: randomized, double-blind, placebo-controlled trials in each MTHFR C677T genotype. *J Hum Genet* **50**, 241–248.
- Muir SR, Collins GJ, Robinson S, Hughes S, Bovy A, Ric DV, van Tunen AJ & Verhoeyen ME (2001) Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. *Nat Biotechnol* **19**, 470–474.
- Nugent AP (2005) LIPGENE: an EU project to tackle the metabolic syndrome. *Biochimie* **87**, 129–132.
- Page T, Griffiths G & Buchanan-Wollaston V (2001) Molecular and biochemical characterization of postharvest senescence in broccoli. *Plant Physiol* **125**, 718–727.
- Paine JA, Shipton CA, Chaggar S, *et al.* (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nat Biotechnol* **23**, 482–487.
- Pareek CS, Czarnik U, Zabolewicz T, Pareek RS & Walawski K (2005) DGAT1 K232A quantitative trait nucleotide polymorphism in Polish Black-and-White cattle. *J Appl Genet* **46**, 85–87.
- Rasinpera H, Savilahti E, Enattah NS, Kuokkanen M, Totterman N, Lindahl H, Jarvela I & Kolho KL (2004) A genetic test which can be used to diagnose adult-type hypolactasia in children. *Gut* **53**, 1571–1576.
- Reynolds VL (2005) Applications of emerging technologies in toxicology and safety assessment. *Int J Toxicol* **24**, 135–137.
- Roy R, Ordovas L, Zaragoza P, Romero A, Moreno C, Altarriba J & Rodellar C (2006) Association of polymorphisms in the bovine FASN gene with milk-fat content. *Anima Genet* **37**, 215–218.
- Saris WH & Harper A (2005) DiOGenes: a multidisciplinary offensive focused on the obesity epidemic. *Obes Rev* **6**, 175–176.
- Saw SM, Yuan JM, Ong CN, Arakawa K, Lee HP, Coetzee GA & Yu MC (2001) Genetic, dietary, and other lifestyle determinants of plasma homocysteine concentrations in middle-aged and older Chinese men and women in Singapore. *Am J Clin Nutr* **73**, 232–239.
- Schaefer EJ, Lamon-Fava S, Ausman LM, Ordovas JM, Clevidence BA, Judd JT, Goldin BR, Woods M, Gorbach S & Lichtenstein AH (1997) Individual variability in lipoprotein cholesterol response to National Cholesterol Education Program Step 2 diets. *Am J Clin Nutr* **65**, 823–830.
- Seshadri S (2006) Elevated plasma homocysteine levels: Risk factor or risk marker for the development of dementia and Alzheimer's disease? *J Alzheimers D* **9**, 393–398.

- Sibley E (2004) Carbohydrate intolerance. *Curr Opin Gastroenterol* **20**, 162–167.
- Slade AJ & Knauf VC (2005) TILLING moves beyond functional genomics into crop improvement. *Transgenic Res* **14**, 109–115.
- Solanky KS, Bailey JC, Beckwith-Hall BM, Davis A, Bingham S, Holmes E, Nicholson JK & Cassidy A (2003) Application of biofluid IH nuclear magnetic resonance-based metabonomic techniques for the analysis of the biochemical effects of dietary isoflavones on human plasma profiles. *Anal Biochem* **323**, 197–204.
- Solanky KS, Bailey NJ, Beckwith-Hall BM, Bingham S, Davis A, Holmes E, Nicholson JK & Cassidy A (2005) Biofluid H-1 NMR-based metabonomic techniques in nutrition research metabolic effects of dietary isoflavones in humans. *J Nutr Biochem* **16**, 236–244.
- Steinhilber SH (2005) Influence of strain and age of hen and dietary fat on the incorporation of omega-3-fatty acids into chicken eggs and on egg quality parameters. *Archiv fur Geflugelkunde* **69**, 94–95.
- Stott DJ, MacIntosh G, Do Lowe G, *et al.* (2005) Randomized controlled trial of homocysteine-lowering vitamin treatment in elderly patients with vascular disease. *Am J Clin Nutr* **82**, 1320–1326.
- Strain JJ, Dowe L, Ward M, Pentieva K & McNulty H (2004) B-vitamins, homocysteine metabolism and CVD. *Proc Nutr Soc* **63**, 597–603.
- Taboada EN, Acedillo RR, Carrillo CD, Findlay WA, Medeiros DT, Mykytczuk OL, Roberts MJ, Valencia CA, Farber JM & Nash JH (2004) Large-scale comparative genomics meta-analysis of *Campylobacter jejuni* isolates reveals low level of genome plasticity. *J Clin Microbiol* **42**, 4566–4576.
- Troen A & Rosenberg I (2006) Homocysteine and cognitive function. *Sem Vasc Med* **5**, 209–214.
- Tuomilehto J, Lindstrom J, Eriksson JG, *et al.* (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* **344**, 1343–1350.
- Ulvik A, Ueland PM, Fredriksen A, Meyer K, Vollset SE, Hoff G & Schneede J (2006) Functional inference of the methylenetetrahydrofolate reductase 677 C > T and 1298A > C polymorphisms from a large-scale epidemiological study. *Hum Genet* Published online: 18 November 2006. doi:10.1007/s00439-006-0290-2.
- van der Werf MJ, Schuren FHJ, Bijlsma S, Tas AC & van Ommen B (2001) Nutrigenomics: Application of genomics technologies in nutritional sciences and food technology. *J Food Sci* **66**, 772–780.
- van Ommen B & Groten JP (2004) Nutrigenomics in efficacy and safety evaluation of food components. In *Nutrigenetics and Nutrigenomics*. *World Rev Nutr Diet*, vol. 93, pp. 134–152 [AP Simopoulos and JM Ordoas, editors]. Basel: Karger.
- Wang YL, Tang HR, Nicholson JK, Hylands PJ, Sampson J & Holmes E (2005) A metabonomic strategy for the detection of the metabolic effects of chamomile (*Matricaria recutita* L.) ingestion. *J Agri Food Chem* **53**, 191–196.
- Weikard R, Kuhn C, Goldammer T, Freyer G & Schwerin M (2005) The bovine PPARGC1A gene: molecular characterization and association of an SNP with variation of milk fat synthesis. *Physiol Genomics* **21**, 1–13.
- Whitfield PD, German AJ & Noble PJM (2004) Metabolomics: an emerging post-genomic tool for nutrition. *Br J Nutr* **92**, 549–555.
- Wild S, Roglic G, Green A, Sicree R & King H (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* **27**, 1047–1053.
- Williams R (2005) Gene tests served up to tell fine foods from fakes. *Nature* **434**, 262.
- Wong GK, Liu B, Wang J, *et al.* (2004) A genetic variation map for chicken with 2.8 million single-nucleotide polymorphisms. *Nature* **432**, 717–722.
- Woolfe M & Primrose S (2004) Food forensics: using DNA technology to combat misdescription and fraud. *Trends Biotechnol* **22**, 222–226.
- Ye X, Al Babili S, Kloti A, Zhang J, Lucca P, Beyer P & Potrykus I (2000) Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* **287**, 303–305.