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Closing Lecture

Plant foods for human health: research challenges

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Plants provide the major part of human food intake. Whilst advances in agronomic characteristics (improved yield and better pest and disease resistance) continue to be a very high priority, there is increasing opportunity to enhance the nutritional value of plant based diets by improving the nutritional quality of staple foods. We now have proof of principle that genetic engineering can be used to produce plant-derived human vaccines. In relation to plant foods for human health, the research challenges include understanding: (1) why certain foods cause adverse reactions in some individuals but not in others; (2) the mechanisms of action of apparently 'protective' foods such as fruits and vegetables. There is also a need to develop much more informative and robust methods for measuring dietary exposure to specific plant foods or food constituents, including both recent exposure, for which a metabolomics approach may be particularly helpful, and long-term exposure.

Plant foods: Genetic engineering: Safety: Metabolomics

Quantity v. quality

Plants provide the major part of human food intake, with the majority of energy being provided by cereals and other starchy staples. Over the past century much of the systematic effort in plant breeding to support the growing human population has focused on increasing the yield per hectare and in protecting crops from pests and diseases. Research to alter the quality of crops, in particular cereals, was designed to improve their utility for processing by, for example, baking or brewing rather than to improve the nutritional value of the product. This approach began to change in the early 1960s with the discovery of a variety of maize (opaque-2) containing a higher concentration of the essential amino acid lysine because of the reduced zein (a water-insoluble prolamine) content (Mertz *et al.* 1964). The potential value of such unusual cereals for those populations subsisting on cereal-rich diets was recognised and nutritional assessment studies were begun (Clark *et al.* 1967). This development stimulated the search for natural diversity, and the use of mutagenesis to create diversity, in the protein concentration and amino acid profile of maize and other cereals. A barley genotype named 'hiproly'

because of its higher content of both protein and lysine (Olsen, 1974) was discovered and research initiated to characterise the amino acid composition of such barley cultivars under different agronomic conditions (Rhodes & Mathers, 1974). More recently, it has been shown that the enhanced protein content and quality of a range of high-lysine barley cultivars are stable when the crops are grown in India (Jood & Singh, 2001).

Although on a gram-for-gram basis these novel cereal cultivars have a higher protein content and an amino acid profile that better matches human nutritional needs, their utility is limited by poor yield and relatively poor agronomic characteristics and, with the exception of work on opaque-2 derivatives of maize, the potential promise for improved human nutrition has not been realised. Over the past 40 years researchers at the International Maize and Wheat Improvement Centre in Mexico and their collaborators have used conventional and novel molecular genetic approaches (Gibbon & Larkins, 2005) to develop maize cultivars (known as quality protein maize) that have up to twice the lysine and tryptophan content in the grain when compared with conventional varieties, without the yield penalty. Some of this developmental work has been

controversial (Crow & Kermicle, 2002), not least because of the debate around the protein (and amino acid) requirements of human subjects and whether there is a practical need to develop cereals with higher than normal protein and amino concentrations (Millward, 1999; Millward & Jackson, 2004).

More recently there has been a push to improve the micronutrient content of staple crops by conventional plant breeding (Welch & Graham, 2004) and by genetic engineering (Ye *et al.* 2000). Given that more than three billion of the world population are micronutrient deficient, such breeding programmes offer a potential low-cost sustainable solution to this problem that may be easier to implement than other strategies, e.g. food fortification or supplementation. However, uncertainties over the bioavailability of the micronutrients in these 'biofortified' crops (Welch & Graham, 2004) and scepticism among substantial numbers of the public about the safety of genetically-engineered food crops remain challenges to be addressed.

Plants as sources of vaccines

The World Health Organization (1996) has estimated that globally approximately two billion (one-third) of the population have been infected with hepatitis B virus, with 350 million suffering chronic infection. Despite the availability of safe and effective injectable vaccines for about two decades, hepatitis B virus infection causes up to one million deaths and >500 000 cases of liver cancer annually (Lai *et al.* 2003). Although more than ninety countries are implementing universal vaccination of newborns against the hepatitis B virus (Lai *et al.* 2003), the requirements for cold storage and the relatively high cost of the vaccine mean that conventional vaccination is more difficult in the remoter areas of developing countries. An alternative approach is to engineer a food crop to express the hepatitis B surface antigen, which might be used for oral vaccination.

The principle of using plants to produce and deliver vaccines was established a decade ago (Haq *et al.* 1995). Plant-based vaccines have been considered to have some unique advantages including: (1) lower costs by utilising conventional crop husbandry methods; (2) reduced potential for adverse reactions, since the transgenic plant is engineered to express only a small antigenic portion of the pathogen; (3) reduced concerns about viral or prion contamination, since no human pathogen is able to infect plants; (4) greater stability of the vaccine, reducing the need for refrigeration; (5) the potential for multi-component vaccines; (6) the possibility of direct oral administration (Streatfield *et al.* 2001). More recently, Tacket *et al.* (2004) have demonstrated that consumption of transgenic maize engineered to express a gene encoding the B subunit of *E. coli* heat-labile enterotoxin results in rises in the serum IgG anti-enterotoxin in healthy human adults. The transgenic maize was administered as uncooked defatted maize-germ meal and repeat dosing was found to increase the immune responses (Tacket *et al.* 2004).

Using a similar strategy Thanavala *et al.* (2005) have engineered potatoes to express the hepatitis B surface antigen. Portions (100–110 g doses) of the peeled raw transgenic potato were fed to healthy volunteers on two to three occasions at 2–4-week intervals. All volunteers had been immunised against hepatitis B previously. Ten of sixteen volunteers (63%) who received three doses of the engineered potatoes were reported to show marked increases in their anti-hepatitis B antibody titres compared with their individual pretreatment values and increases in titres with both time and number of doses (Thanavala *et al.* 2005). These responses to oral vaccination were obtained without the use of an adjuvant, and the authors suggest that both the magnitude and rate of response might be increased by the use of mucosal adjuvants in future studies (Thanavala *et al.* 2005). Whilst this study provides proof of principle of the use of transgenic plants to synthesise and deliver an edible vaccine for hepatitis B, the current approach has a number of limitations. First, this approach is unlikely to be suitable for initial vaccination (conventional injectable vaccine will still be needed) but it could replace the need for repeated booster injections to maintain immunity. This procedure would have particular advantages where access to the conventional vaccine is limited and where there is a high risk of infection with, for example, HIV from re-use of needles. A second limitation is the need to consume the plant vehicle, in this case potatoes, in the raw state to avoid heat damage to the vaccine in cooking. This requirement suggests that transferring the technology to tomatoes or other foods that are normally eaten raw would be much more attractive to potential recipients of the vaccine, but this approach would bring new problems in terms of storage and transport of the engineered food, which are less of an issue for cereal grains such as maize (Tacket *et al.* 2004).

Safety of GM food crops

Molecular genetic and other molecular biological tools are essential components of any modern research programme attempting to understand the biology of food crops and develop crops with improved qualities (Gibbon & Larkins, 2005). Among the first generation of GM food crops were those engineered to be resistant to herbicides, e.g. glyphosate-resistant varieties of soyabean (Padgett *et al.* 1995), and those with enhanced resistance to pests, e.g. maize strains that express *Bacillus thuringiensis* δ -toxin (Kozziel *et al.* 1993). In assessing the safety of such foods for human consumption it is important to know whether the transgene inserted into the novel crop could survive within the human intestine, whether it might be taken up by the gut microflora and incorporated into the bacterial genome or whether there is horizontal gene transfer to enterocytes or other human cells. As a first step in investigating these possibilities, the degradation of transgenic DNA from GM soyabean and maize during *in vitro* simulations of human intestinal digestion has been quantified (Martin-Orúe *et al.* 2002). The transgene in naked DNA (extracted from GM soyabean), but not the DNA within GM soyabean, was found to be rapidly degraded when exposed to gastric

simulations (80% disappeared within 60 min). Simulation of small intestinal digestion results in degradation of the transgenes in both GM maize and GM soyabean, but after 3 h approximately 5% (soyabean) and 3% (maize) of the initial quantity of transgene remains detectable (Martin-Orúe *et al.* 2002). These *in vitro* data suggest that some transgenes in GM foods could survive passage through the stomach and small intestine with the potential for horizontal gene transfer. The next step in the investigation was to assess the survival of transgenic DNA in the human small intestine (Netherwood *et al.* 2004). After an overnight fast healthy subjects with ileostomies were given a test meal consisting of GM-soya burgers and a GM-soya-milk shake and the contents of their stoma bags were collected every 30 min for 6 h for estimation of transgene survival through the small bowel. The transgene could be quantified in digesta from all seven volunteers in amounts equivalent to $\leq 3.7\%$ of the dose in the original test meal. To assess the fate of the transgene in the large bowel the same test meal (with the inclusion of radio-opaque markers ('Colon Transit 20')) was fed to twelve healthy volunteers (with intact gastrointestinal tracts) and stools collected for 72 h. For all volunteers 90–98% of the indigestible marker was recovered in faeces but the GM soyabean transgene could not be detected (Netherwood *et al.* 2004). This result is evidence that although a proportion of the copies of the transgene can survive passage through the small bowel, it is degraded completely in the large bowel. In this respect, it is probable that the transgene does not behave differently from other plant DNA, since the recovery of the transgene in ileal effluent was found to be highly correlated with that of the soyabean lectin gene *Lel* (Netherwood *et al.* 2004).

Intriguingly, the soyabean transgene was found to be detectable in bacteria cultured from the baseline ileostomy effluent, i.e. before consuming the test meal, for three of the seven volunteers (Netherwood *et al.* 2004). These data suggest that the volunteers had consumed the transgene before enrolling in the study and that horizontal gene transfer from GM food to the intestinal microflora had occurred. This gene flow is likely to be the result of long-term exposure to GM foods (Netherwood *et al.* 2004). The consequences, if any, for gastrointestinal function or for the function of the intestinal bacterial that have taken up the transgene are unknown. However, whilst there is no *a priori* reason to suppose that such horizontal gene transfer events pose a risk to the health of the human consumer of such GM foods, it will be prudent to explore this issue further. For example, it will be important to identify the organism(s) that have taken up the transgene and to determine where it has integrated into the bacterial genome. This information may provide clues to the characteristics of transgenes that make them more susceptible to horizontal gene transfer. Based on mathematical modelling, other researchers have argued that the rare bacterial transformants that have acquired transgenes by horizontal gene transfer will require years of growth to out-compete wild-type bacteria and that the current monitoring approaches are inadequate for the task of verifying or falsifying the horizontal gene transfer hypothesis (Nielsen & Townsend, 2004).

Recent studies have failed to detect the transgene in the blood of chickens fed with *Bacillus thuringiensis* δ -toxin-containing maize (Rossi *et al.* 2005) or in the tissues of piglets fed a GM (MON810) maize (Mazza *et al.* 2005), leading the authors to conclude that it is 'unlikely that the occurrence of genetic transfer associated with GM plants is higher than that from conventional plants'.

Plant foods and human health

For most of the common disorders and diseases that afflict man, including obesity, type 2 diabetes, CVD and many cancers, diets that are rich in fruits and vegetables are associated with lower risk (World Health Organization/Food and Agriculture Organization, 2003). There is also evidence that diets rich in plant foods may increase longevity. For example, using a cohort of 76 707 men and women aged ≥ 60 years from nine countries participating in the European Prospective Investigation into Cancer and Nutrition (the EPIC-elderly Study), Trichopoulou *et al.* (2005) have reported that those subjects who had a higher score for adherence to a Mediterranean diet had a lower risk of mortality. The diet score ranged from 0 to 9 and a two-unit increase in diet score was found to be associated with an 8% decrease in mortality (Trichopoulou *et al.* 2005). Those subjects adhering to the Mediterranean diet were found to have high intakes of vegetables, fruits and cereals, moderate to high intake of fish, low meat intake, low intake of saturated fatty acids, high intake of MUFA (olive oil), low to moderate intake of dairy products (principally cheese and yoghurt) and moderate intake of ethanol (mostly wine; Trichopoulou *et al.* 2005). Although the association was found to be stronger for subjects from Greece and Spain, no statistically significant heterogeneity among countries in the relationship between diet score and mortality was found, suggesting that the modified Mediterranean diet is beneficial to health across countries (Trichopoulou *et al.* 2005).

Mechanisms of action

Genomic damage resulting in aberrant gene expression is a fundamental step in the development of much pathology, and plant-derived bioactive food components may protect against such pathology by intervening in the pathways that regulate gene expression (Milner, 2004). Since both external agents and normal cell functions, such as mitosis, subject the genome to frequent and diverse insults, the human cell has evolved a battery of defence mechanisms that attempt to minimise such damage (including inhibition of oxidative reactions by free radical scavenging and the detoxification of potential mutagens), to repair the damage or to remove severely-damaged cells by shunting them into apoptosis (Mathers 2004a). All these processes are potentially modifiable by components in foods and by nutritional status (Steele, 2003) and may help explain why diets rich in plant foods appear to be protective against a wide range of common diseases (World Health Organization/Food and Agriculture Organization, 2003).

Much of the work in this area has focused on micro-nutrients such as vitamins A, C and E, and trace minerals including Zn, Se and Mn that are believed to prevent oxidative damage (antioxidants; Ames & Wakimoto, 2002). However, intervention trials have failed to provide unequivocal evidence that antioxidant supplements will reduce disease risk. Indeed, major intervention studies in which middle-aged male smokers (or those exposed previously to asbestos) were given high doses of β -carotene have shown no protective effect against cancer. Worse, a greater incidence of lung cancer was found among those subjects supplemented with β -carotene (Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994).

The genome in every cell is exposed continually to damage by both exogenous and endogenous factors, and man has evolved a range of DNA repair mechanisms that sense and repair different kinds of DNA damage. For example, signals from both oxidative stress and DNA strand breaks are integrated by p53 (Liu & Kulesz-Martin, 2001) leading to cell cycle arrest (to allow time for DNA repair) or to apoptosis (Mathers, 2003). The major types of DNA repair in mammalian cells include one-step repair, base excision repair, nucleotide excision repair, DNA mismatch repair and recombinational repair. Recent research suggests that dietary antioxidants may protect the cell through up regulating DNA repair (Brash & Havre, 2002). Importantly, there is now evidence from a human intervention trial that improved nutrition may enhance DNA repair in human subjects *in vivo*. When the diet of healthy human volunteers was supplemented with kiwi fruit (*Actinidia deliciosa*) for 3 weeks (Collins *et al.* 2003) reduced endogenous oxidation of purines and pyrimidines and enhanced base excision repair by lymphocytes were observed. The molecular mechanisms for the enhanced base excision repair in those consuming supplemental kiwi fruit were not elucidated in this study, but these exciting observations should encourage investigation of the underlying mechanisms and the potential for enhancement for each DNA repair mechanism by factors in plant foods. Tentative evidence that low folate status may limit the cell's ability for nucleotide excision repair has emerged recently from a human epidemiological study (Wei *et al.* 2003), suggesting that there may be enhanced capacity for DNA repair in those individuals consuming diets rich in fruits and vegetables with greater folate intake.

Measurement of exposure to plant foods

Characterisation and quantification of dietary exposure in epidemiological and other studies is very challenging because of the complexity of eating behaviour, the wide range of consumed foods, the heterogeneity of consumption between individuals and within individuals over time and the errors inherent in all available tools for dietary assessment. The challenge is even greater if the objective is to compute exposure to specific food constituents because of the chemical complexity of foods, the limitations imposed on food composition tables by frequent changes in the raw materials used in food processing and

the lack of information on the digestion, absorption and metabolic transformation of most food constituents, especially non-nutrient food constituents. Attempts have been made to develop objective measures of some aspects of dietary exposure using, for example, concentrations of substances in blood, urine or other body tissues or fluids (Bingham, 2002; Ross *et al.* 2004), but in the main these measures have been designed to validate recordings of dietary intake made by study participants. For example, Bingham *et al.* (1997) have compared 24 h urinary N and K excretion and serum vitamin C and carotenoid concentrations with intakes estimated from a variety of dietary assessment methods.

The rapidly-evolving science of metabolomics offers the possibility to develop novel approaches that could be capable of characterising and quantifying the totality of dietary exposure and would reduce, or eliminate, the need to rely on subjective reporting by study volunteers. Metabolomics is the study of all the low-molecular-weight molecules present in cells, tissues or biofluids using instrumentation, including NMR spectrometry and advanced one- and two-dimensional MS (Gibney *et al.* 2005). This approach may be particularly useful for estimating exposure to plant foods, since in comparison with foods of animal origin plant foods contain a wide range of characteristic secondary metabolites (Acamovic & Brooker, 2005). As yet, the application of metabolomics approaches to the study of intakes of individual foods or of the whole diet is in its infancy and faces several challenges, not least the need for the development of comprehensive datasets of identified metabolites present in human body fluids. The ambition to develop metabolomics-based protocols for characterising dietary exposure to plant foods is likely to drive research on: (1) the very large number (tens of thousands) of plant metabolites (the plant metabolome); (2) the metabolites present in such foods after cooking (the food metabolome); (3) the digestion (including large-bowel fermentation) and absorption of plant-derived metabolites (the absorbed metabolome); (4) the metabolism of absorbed moieties to generate the metabolites present in blood (the plasma metabolome); (5) metabolites excreted in urine (the urinary metabolome).

Whilst the metabolomics approach may help to characterise current dietary exposure, it is unlikely to be able to address the much bigger challenge of detecting and quantifying dietary exposures some time in the past or over a prolonged time period, which would be of particular use in epidemiological studies. Obtaining this information would require the identification of processes through which environmental exposures are recorded and remembered. It has been shown recently that chemical modifications (including acetylation, methylation, phosphorylation, ubiquitination and ADP-ribosylation) of the tails of histone proteins (the octet of globular proteins around which DNA is wrapped within the nucleosome) occur in response to changes in the environment of the cell, including the nutritional environment (Oommen *et al.* 2005). Moreover, it is becoming apparent that it is through such changes in epigenetic markings that the genome integrates exposure to both intrinsic and extrinsic signals resulting in altered gene expression (Jaenisch & Bird, 2003). As changes in

epigenetic markings are copied across cell generations, this process offers a potential avenue to develop novel approaches to the tracking of nutritional exposure over prolonged time periods (Mathers, 2004b).

Concluding comments

In their 20-year vision paper *Plants for the Future*, the European Genval Group of plant scientists, industry representatives and European Commission representatives (European Commission, 2004) have concluded that there is a strategic priority to improve the safe exploitation of the genetic diversity in plants to 'produce better quality, healthy, affordable, diverse food offering consumers in and beyond Europe real options to improve their quality of life'. This document highlights the potential benefits of the application of genomics and other biotechnological approaches in 'breeding by design' novel food crops with enhanced health benefits and, in particular, foods that encourage healthy choices to help reduce the risk of heart disease, cancer and obesity, and foods to help maintain health in older individuals. However, a major impediment in achieving this aim is the 'political inertia caused by the polarised and increasingly heated debate between opponents and advocates, with a sceptical and confused public caught in the crossfire' (European Commission, 2004). Alongside the clear recognition that plant breeders and the biotechnology companies that exploit their work need to focus more on the benefits of their science for the consumer, there is a need for further research that tests the assertion that GM foods are safe and may therefore go some way to reassuring the public.

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