International recognition of functional foods has resulted in the recent global development of this field, which originated in Japan. The national policy on functional foods, in terms of “foods for specified health use”, has also been developing and has motivated the food industry to produce a variety of new food items. In Japan as well as in many other countries, academic and industrial scientists have been working in collaboration for the analysis and practical applications of functional food science. Emphasis has been placed on the study of antioxidant and anticarcinogenic food factors as well as pre- and probiotics. This review pinpoints recent trends in the science and industry in this field.

Key words: functional foods; oxidative stress; anticarcinogens; prebiotics; probiotics

The term “functional food” (in English) first appeared in Nature (1993) with the headline “Japan explores the boundary between food and medicine”. Neither the terminology nor the concept existed until nine years before that. In 1984, an ad hoc research group began to implement a systematic, large-scale national project under the sponsorship of the Ministry of Education, Science, and Culture (MESC) to explore the interface between medical and food sciences. The realization of such a novel field of health science in Japan may be the result of underlying traditions, expressed in the ancient Chinese saying, “Medicine and food have a common origin”.

The MESC research project was followed by policy development. In 1991, the Ministry of Health and Welfare (MHW) initiated a world-first policy by legally approving the commercialization of selected functional foods in terms of “Foods for Specified Health Use” (FOSHU). The policy was defined by new legislation and involves permitting a health rebate for use of each FOSHU product. This legal framework is intended to prohibit ill-defined or misleading advertisement of commercial products. Many FOSHU products have appeared on the market since the first approval of hypoallergenic rice. Such a trend gave a strong impetus to the food industry in Japan, by motivating scientists and technologists to design and produce foods with improved and beneficial functionalities.

These scientific, political, and industrial developments in the field of functional foods have had a strong impact on many nations, particularly in Europe. In 1995, the U.K. government defined, albeit temporarily, functional foods as those that have had components incorporated to provide specific medical or physiological benefits other than nutritional effects. The European body of the International Life Science Institute (ILSI Europe) has taken special interest stating that “we stand today at the threshold of a new frontier in nutritional science”. The concept of food has been changing from its past emphasis on eating to prevent hunger into the potential use of foods to reduce the risk of chronic illness. This new concept is important in view of the need of elderly populations for improved quality of later life.

As recognized in the Japanese MESC research project, up-to-date knowledge in biochemistry and
molecular biology supports the hypothesis that foods can modulate various functions in the body and thus participate in the maintenance of a state of health that reduces the risk of lifestyle-related diseases. It is such an hypothesis that is the platform for the concept of functional food and the development of a new discipline, functional food science, in Europe as well as in Japan. What is most important at present is to evaluate a food’s functions on the basis of scientific evidence. A key approach to developing a functional food is the identification and validation of relevant markers, including biomarkers, that can predict potential benefits related to a target function in the body. If the markers are of an event directly involved in the process, these should be considered as functional factors. On the other hand, if the markers represent correlated events, they should be considered to be indicators. Possible markers have been tentatively listed in relation to target functions.5) There are many options for selection of appropriate markers. One promising option may be the use of accumulated knowledge regarding genomics. As soon as the human genome project is completed, the so-called post-genomics era will begin, in which a number of sophisticated technologies will become available, such as DNA chip technology.6) The high throughput of this technology will allow assessment of the possible effects and safety of functional foods from total gene expression profiles.

With this as background, the ILSI Europe organized an international symposium in Paris, October 17–19, 2001, entitled “Functional foods—scientific and global perspectives”. Some 340 people, most from universities, companies, and governments, participated and contributed to very lively discussions. One of us (S.A.) was invited as the Asian representative and delivered a keynote speech dealing with global views on functional foods. Proceedings of the symposium will be published elsewhere.

In Japan, the scientific, industrial, and political development of functional foods is a matter of great public interest. The first symposium (2000) sponsored by the Japanese branch of the International Union of Food Science and Technology (IU FoST-Japan), entitled “A mainstay of functional food science in Japan”, was enthusiastically attended and the outcomes have been reviewed.7) In 2001, the second IU FoST-Japan symposium on the latest trends in functional food science and industry was organized by another of us (S.K.) and held in Tokyo, with nearly 300 attendees gathering, mostly from universities and companies. Leading scientists in the field of functional foods described their current projects and contributed to discussions of mutual interest. Details are reported here to make available internationally information about the status and the importance of this new category of foods.

### Table 1. International Market for Functional Foods in Selected Countries and Regions, 1999

<table>
<thead>
<tr>
<th>Country</th>
<th>Strict definition ($ billion)</th>
<th>Broad definition ($ billion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>1.79</td>
<td>4–8</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>1.80</td>
<td>15</td>
</tr>
<tr>
<td>Japan</td>
<td>2.13</td>
<td>14</td>
</tr>
<tr>
<td>Australia</td>
<td>0.05</td>
<td>na</td>
</tr>
<tr>
<td>Total</td>
<td>5.77</td>
<td>&gt;33</td>
</tr>
</tbody>
</table>

na, Not available.  
Source: M. Hilliam, The World of Food Ingredients, December 2000.5)

### Global trends

Extensive research on the relationship between food and health has opened the door for functional foods. The concept of functional food has now become one of the most important keywords in the food industry worldwide. The functional food market is undoubtedly expanding in most countries that have an established processed food market.

Estimation of the size of the functional food market is difficult because the understanding and adoption of functional food is different in different regions of the world. Table 1 shows the estimated values of the international market for functional foods in 1999.8) When applied in its strictest definition, limiting the analysis to foods (including drinks) that have health claims on their packaging or in their advertising material, the combined value of the functional food market in Europe, the U.S.A., and Japan is some $5.7 billion. When a broader definition of the market is used, the combined value is estimated at over $33 billion.

In the U.S.A., functional food is broadly defined as neutraceuticals, including dietary supplements. The focus is on reduction of the risk of cancer and coronary heart disease through strengthening of the immune system and reducing cholesterol levels, respectively. Following the designer food programs of the past, commercialization of functional food has continued to the present. Taking the broader definition, the U.S. retail market for functional foods (excluding dietary supplements) was estimated to be around $14.8 billion in 1998 and projected to grow from 12 to 15 percent annually through 2003.9) The main reasons for this rate of growth are: 1) the increased income of consumers because of a wave of economic prosperity and increased investment by enterprises, 2) the establishment of the Dietary Supplement, Health and Education Act (DSHEA), and 3) an efficient distribution network. In 1999, two kinds of cholesterol-reduction spreads were launched on the U.S. market, one after the first. One was “Take Control” (Lipton/Unilever), which contains plant sterol esters. The other was “Benecol” (McNeil/Johnson & Johnson), which was developed by the
Finnish company Raisio and contains stanol esters (a hydrogenated sterol from wood pulp). The US-FDA approved heart health claims for plant stanol in September 2000. The launch of cholesterol-reduction spreads created much interest in the heart-health area. Consequently, soy has had much attention following the FDA’s approval of a soy protein-heart health claim in 1999. Soy-milk and soy-cake (tofu), originally from Japan, as well as the soy protein cereals such as “Smart Start” (Kellogg) are recent successes as functional foods. Bone health and cancer prevention are other areas of interest in the U.S., but gastrointestinal health has not raised the same level of interest there as in Europe or Japan. Despite the activity of the functional foods market, R&D investment in functional foods seems to be small in the U.S., perhaps because there is an emphasis on medical research, where returns on the investment are better defined. Consequently, research on functional foods is inconspicuous except for phytochemicals such as isoflavon and polyphenols that are expected to be effective for heart health and cancer prevention.

In Europe, the key area for functional foods is gut health with probiotic dairy products. The interest of European countries in functional foods was modest until the mid-1990s. However, after the launch of a probiotic yogurt, “LC1”, by Nestle in 1994, the functional foods market increased. Danone also launched a probiotic yogurt, “Actimel”, soon after Nestle. The European functional foods market, by the strict definition, was estimated at $1.8 billion in 1999, and by the broader definition, was estimated at $4 to 8 billion.8) The European market of functional dairy products in 1999 was estimated to be at 1.34 billion Euros.8)

In response to market expansion, the European Commission’s “Concerted Action” on Functional Food Science in Europe (FUFOS) began in 1995. The Concerted Action was coordinated by ILSI Europe (International Life Science Institute, Europe Branch) and supported by the European Administration with the aim to establish a science-based approach for concepts in functional food science.10) The goal of this concerted action has been to set up a multidisciplinary European network: 1) to assess critically the science base needed to examine evidence that specific nutrients and food components positively affect target functions in the body, 2) to examine the available science from a function-driven perspective rather than a product-driven one, and 3) to reach consensus on the targeted modification of food constituents and gather opinions regarding their application. The contents of the third general assembly of FUFOS in November 1998 was published as “Scientific Concepts of Functional Food Science in Europe: Consensus Document”.10) After that, the concerted action continued so the conceptual work was converted into research practice. Some of the results of the ensuing research were reported at the International Symposium on Functional Foods (Paris, 2001).

In China, there is an approval system for “Foods for Maintaining Health” equivalent to that of the FOSHU (Food for Specified Health Use) system in Japan. At present, approximately 2,000 food items have been approved.11) The foods are sold at the same kind of places as general processed foods, including large grocery stores in China. This category includes products such as capsules or tablets and also as more general food forms such as biscuits or candy. The effective constituents include not only natural remedies, such as traditional ginseng, but also functional food materials such as EPA and DHA that are of interest around the world. A total of 24 favorable effects on human health have been officially authorized for food labels after the food receives approval. They include immune-regulation, anti-aging, memory improvement, obesity reduction, and anti-cancer effects.

The desire of people to remain healthy together with manufacturers’ desire to add value to their products may not diminish. Probably, research into functional foods will increase and the market size will continue to expand globally.

Analysis of antioxidant foods

Mechanisms for antioxidant effects are complicated, and elucidation has been difficult given conventional experimental systems. Currently, a system using the latest technology for the screening of food components to identify candidate cell signaling pathways of antioxidants or other factors is being built. The goal may be achieved by a combination of DNA chip technology and the Cell Signaling Network Database (CSNDB), maintained by the National Institute of Health Sciences.12)

Many antioxidant factors derived from foods have been identified by the EB virus test in Raji cells,13,14) and the effects of antioxidants have been confirmed mostly by the mouse skin test in vivo.15,16) However, gene analysis associated with the EB virus test with Raji cells has not been fully developed.

The DNA chip microarray technique, which has rapidly advanced in recent years, allows systematic screening of gene expression. Previous experiments on aging and apoptosis of cultured cells that used microarrays has provided useful data. DNA chips may become the core technique facilitating genome research in the EB virus test, but difficulties in reciprocal comparison and interpretation of data are still obstacles to the study. Therefore, the effects of retinoic acid, which is one of the best known antioxidant food factors, on gene expression on Raji cells after treatment with 12-O-tetra-decanoylphorbol-13-acetate (TPA) has been investigated. In addition, as a
one-time-only trial, a large amount of data in DNA chips with the cell signaling network data base (CSNDB) developed at the National Institute of Health Sciences were compared, and analysis of the role of genes in intracellular signal transmission was undertaken. In such a way, antioxidants derived from foods can be screened by a combination of DNA chip technology and CSNDB.12)

Retinoic acid, a typical antioxidant derived from foods, and an early antigen inhibitor are used. Because retinoic acid is expected to have applications in medicine, such as differentiation induction therapy for leukemia, it was selected as a substance with clinical potential. Retinoic acid has important roles in regulating genes involved in cell morphogenesis, differentiation, and proliferation. A variety of functions of retinoic acid may explain its dramatic non-antioxidant effects in vivo. Retinoid-responsive genes are complex. A study in which a large number of genes were simultaneously assayed by DNA chip analysis was due to identify genes according to function or signal transmission with use of the CSNDB program. The CSNDB program contains a variety of information about signal pathways. Molecules involved in physiological dynamics are included in the form of GeneBank AC numbers, but it should be noted that the CSNDB program is still under construction and is not yet fully comprehensive.

Another of the trial studies is to investigate how to identify candidate pathways efficiently from enormous pools of gene expression data. This trial may be invaluable for DNA chip-microarray users because of the new methodology of combined array data and database information. Preliminary data have been entered into the CSNDB program and so far three candidate pathway have been found. It seems that genes regulated in Raji cells by retinoic acid may play a role cell differentiation by various mechanisms that may become therapeutic targets with a view to pharmacogenomics. In this strategy, useful functional food factors are being sought by the identification of candidate pathways of various effects (Fig. 1). Rapid and simultaneous analysis of multiple parameters and their interaction with computer data bases, as is due in these studies, may elucidate complex biosystems and provide clues that will facilitate not only the medical and pharmaceutical fields but also drug exploration in various other fields.

Development of antioxidant foods—catechin, sesamin and arachidonic acid

For the role of dietary antioxidants to be ascertained, it is necessary to understand the chemical nature of the forms absorbed into the circulation and how research findings in vitro reflect the bioactivity of antioxidants in vivo.

It is widely accepted that polyphenols in food are powerful antioxidants in vitro, but their active structures in vivo have yet to be clarified. (+)-Catechin and (−)-epicatechin are biologically effective antioxidants, found particularly in red wine and tea. The metabolism of these compounds was investigated so that their active structures in biological fluids after oral administration to rats could be identified. Without prior treatment of plasma with β-glucuronidase and sulfatase, two metabolites was detected at much higher concentrations in the plasma, bile, and urine than in the concentration of the originally ingested compound. Each major metabolite found in the plasma at the highest concentration was excreted into both the bile and urine as well, and was purified from the urine. Their chemical structures were found to be (+)-catechin 5-O-β-glucuronide and (−)-epicatechin 5-O-β-glucuronide by MS and NMR analyses. These glucuronide conjugates had high antioxidative activity as superoxide anion radical scavengers, as did their parent compounds. It was concluded that (+)-catechin 5-O-β-glucuronide and (−)-epicatechin 5-O-β-glucuronide are the biologically active structures in vivo of the ingested polyphenolic antioxidants.17)

First, the radical-scavenging effect of sesamin in

Fig. 1. Screening of Antioxidant Foods by DNA Chip Technology.
rats was examined with an L-band electron spin resonance spectroscopy (ESR).

Sesamin is a lignan present abundantly in sesame seed. A series of biological activities of sesamin have been reported to date, including protective effects against liver damage caused by alcohol or CCl₄ in rodents, suppressive effects against 7,12-dimethylbenzo[a]anthracene-induced rat mammary carcinomaogenesis, suppression of lipid peroxidation in rats and in humans, and inhibition of the development of preneoplastic foci induced by diethylnitrosamine in rat liver. Antioxidant activity was proposed as an explanation of these effects of sesamin but the explanation has not been proven. Next, the antioxidant role of sesamin in rats both in vivo and in vitro was investigated.

Rats were given sesamin orally as a suspension in olive oil. 4-Hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL) was injected into the tail vein 3 h after sesamin treatment. ESR spectra obtained with L-band ESR with a flexible surface-coil-type resonator were obtained from the liver, kidneys, and inferior vena cava of rats under anesthesia after the injection. Oral administration of sesamin caused more TEMPOL to be eliminated than with the control group. However, this effect occurred only in the liver, not in the kidneys or inferior vena cava. Sesamin has radical scavenging effects only in the liver. ²⁷

Sesamin was given orally to rats, and their blood, bile, and urine were sampled periodically. More than 40% of the total dose of sesamin was detected in bile (but not in blood or urine) as glucuronides of 2-(3,4-methylenedioxyphenyl)-6-(3,4-dihydroxyphenyl)-cis-dioxabicyclo[3.3.0]octane (di-catechol metabolite) and 2-(3,4-dihydroxyphenyl)-6-(3,4-dihydroxyphenyl)-cis-dioxabicyclo[3.3.0]octane (di-catechol metabolite) within 24 h of administration. Antioxidant activities of these catechol metabolites were compared to sesamin; strong radical scavenging activities toward superoxide anion radical was found in mono- and di-catechol metabolite. It is noteworthy that di-datechol metabolite scavenged not only superoxide anion radicals but also hydroxyl radicals (Table 2). From these results, it seemed that sesamin was absorbed via the portal vein in native form and metabolized to the mono- or di-catechol compound by enzymes in the hepatocytes. Both metabolites had antioxidant activity in the liver and were finally conjugated with glucuronic acid by glucuronidase for excretion into the bile. Sesamin has been classified as a pro-antioxidant. ²⁹

Arachidonic acid has double bonds in its structure and therefore can be classified as an antioxidant. This compound is essential for the construction of cell membranes. However, infants do not synthesize arachidonic acid readily and are liable to be deficient. It is now being recommended that arachidonic acid be added to infant formula, which is deficient in this compound.

In 1987, Yamada et al. ²⁰ screened for microorganisms that can accumulate lipids containing arachidonic acid and found one, a filamentous fungus, Mortierella alpina 1S-4. Fatty acid metabolism in this strain has been studied and the results have been applied to the industrial production of arachidonic acid. Recently, the reduction of arachidonic acid content in the brain was observed in aged rats and this correlated well with the reduction of long-term potentiation. It is important to note that both were reversed by the feeding of arachidonic acid. ²¹ Next, it was examined whether memory was preserved in aged rats on an arachidonic acid-enriched diet. Probe test of young (5 months old) and aged rats (22 months old) on the control diet in Morris water-maze after removing the platform showed that memory loss was abundant in aged rats. Arachidonic acid-enriched diet for aged rats was effective for the prevention of this memory loss (Fig. 2). ²² Dietary intake of arachidonic acid increased the arachidonic acid content in the brain and reduced memory loss.

### Analysis of anti-tumor foods

Dietary habits are important factors in both the development of cancer and anti-tumor potential of hosts. Epidemiologic and experimental data suggest that differences in diet contribute to variations in cancer incidence. Anti-tumor foods are divided into two categories: cancer prevention and cancer therapy. During the early stages of anti-carcinogenesis, DNA damage may be prevented by antimutagens (anticarcinogens) in some foods. Induction of phase II detoxification enzymes (e.g., glutathione transferases) is

<table>
<thead>
<tr>
<th>Table 2. Antioxidant Activity of Sesamin and Its Metabolites</th>
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<tr>
<td>Antioxidant activity (%)</td>
</tr>
<tr>
<td>O₂&lt;sup&gt;-&lt;/sup&gt; ²¹</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Sesamin</td>
</tr>
<tr>
<td>Mono-catechol</td>
</tr>
<tr>
<td>metabolite</td>
</tr>
<tr>
<td>Di-catechol</td>
</tr>
<tr>
<td>metabolite</td>
</tr>
</tbody>
</table>

1) Superoxide anion radical (O₂<sup>-</sup>) scavenging activity was analyzed by ESR at 50 μM, using 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) spin adduct generated by the hypoxanthine and xanthine oxidase reaction.
2) Hydroxy radical (·OH) scavenging activity was analyzed by ESR at 250 μM, using DMPO spin adduct generated by the Fenton reaction.
3) Inhibitory activity of lipid peroxidation was measured by the thiobarbituric acid-reactive substance (TBARS) method at 60 μM.
and IFN-γ-stimulated with phytohemagglutinin for 24 h. IL-12 is an essential cytokine that induces T helper Th1 cells from naive cells (Th0). The production of IL-12 decreases with the progress of the cancer. (Th) 1 cells from naive cells (ThO).

In bacterial infections, the overproduction of TNF-α may cause septic shock leading to acute organ failure and death. TNF-α is a mediator in skin diseases such as the contact hypersensitivity reaction. The inhibition of TNF-α overproduction is therefore necessary for the cure of chronic inflammatory disease, including carcinogenesis.

The overproduction of TNF-α has been suppressed by orally administering a perilla leaf extract, \(^{26}\) whatever the strain tested. The inhibitory activity against TNF production was heat-stable, and the existence of several active molecules was suggested. When perilla leaf was extracted with solvents, the strongest activity was in the aqueous preparation, although some activity was detected in preparations extracted with n-hexane and ethyl acetate. These findings suggest that the daily use of certain functional foods may be useful for controlling the host defense system.23 The oral administration of perilla leaf extract to mice inhibited two acute inflammatory model; arachidonic acid-induced ear edema and 12-0-tetradecanoylphorbol-13-acetate-induced ear edema. Oral administration of perilla leaf extract also inhibited the contact dermatitis model in which ear edema is caused by oxazolone sensitization.

Tumor immunity is mainly affected by cellular immunity, which can be stimulated by certain cytokines such as interleukin (IL)-12 and interferon-γ (IFN-γ). IL-12 is an essential cytokine that induces T helper (Th) 1 cells from naive cells (ThO). The production of IL-12 decreases with the progress of the cancer. Thirty-eight patients with solid tumors were given mushroom extracts orally for 6 months and their peripheral blood was sampled every 2 months for examination of the effects of the extracts on their immune function. Peripheral blood lymphocytes were stimulated with phytohemagglutinin for 24 h. IL-12 and IFN-γ were measured by enzyme-linked immunosorbent assays, and the basal levels of these cytokines in the patients with tumors were lower than those in healthy controls. Immunological parameters of the patients increased to normal levels during intake of the extracts. These results showed that edible-mushroom extracts improved both immunological abnormalities and clinical presentation.25

Chronic inflammation also seems to be capable of giving rise to carcinogenesis. Inflammatory cytokines, which are frequently involved in chronic inflammation, can be inhibited by some non-nutrient foods (e.g., Perilla leaves and green tea) at the site of chronic inflammation, resulting in anti-carcinogenic activity. Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine, originally discovered because of its antitumor activity; it seems to be involved in many immunological and inflammatory reactions. However, excess production may lead to immunological and inflammatory diseases; for example, prolonged exposure to TNF-α might cause cachexia. In bacterial infections, the overproduction of TNF-α may cause septic shock leading to acute organ failure and death. TNF-α is a mediator in skin diseases such as the contact hypersensitivity reaction. The inhibition of TNF-α overproduction is therefore necessary for the cure of chronic inflammatory disease, including carcinogenesis.

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Tumor promotion is a crucial stage in human carcinogenesis. The activation of TNF-α production by chemical tumor promoters is the essential event in tumor promotion. The continuous production of TNF-α is the determining factor in tumor promotion, and inhibition of TNF-α helps to prevent cancer. In fact cancer chemopreventive foods, such as perilla leaves and green tea, which inhibited both tumor promotion and TNF-α production.

For cancers to grow and to spread to secondary sites, they need a blood vessel system for nutrients supply and for removal of waste products. If angiogenesis is inhibited, then the cancer cannot progress at that site. Therefore, products and preparations that are anti-angiogenesis are likely to be useful in the treatment of cancers. Highly concentrated preparations of isoflavone aglycons from soybeans and polysaccharides have anti-tumor activity because of anti-angiogenic activity.

Antitumor foods should be analyzed in terms of the various aspects of carcinogenesis: anti-carcinogens, anti-initiation, induction of phase II detoxification enzymes, anti-promotion, anti-inflammation, anti-oxidative stress, immune stimulation, cytokine production, anti-angiogenesis, intestinal flora, anti-metastasis, cell differentiation, anti-immunodepression, and tumor cytotoxicity. A large number of foods could be candidates for the prevention and therapy of various cancers.

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**Fig. 2. Effects of Arachidonic Acid-enriched Diet on Probe Test in Morris Water-maze after Removing the Platform in Rats.**

Typical paths taken from starting position ("S") to the target quadrant (outlined with dots) learned in the water-maze task. This figure demonstrates the effects of the diet on spatial learning and memory in rats. The data suggest that the arachidonic acid-enriched diet improves spatial navigation and memory consolidation in the Morris Water-maze test.
Development of anti-tumor foods

The contribution of intestinal microflora to facilitating the health of the host has been confirmed in many studies. The concept of probiotics is now widely accepted, so the use of beneficial intestinal bacteria such as lactic acid bacteria is becoming more common for maintenance of healthy and also for prevention and treatment of diseases. Before establishment of this concept, investigations into the microecology of intestinal bacteria from the viewpoint of preventive medicine were undertaken. Specifically, the prevention of cancer not with drugs or other chemical substances but with intestinal bacteria and, in particular, with fermented milk products containing such bacteria has been examined.

The following are subjects of the investigations. The relationship between intestinal bacteria and the genesis or prevention of cancer was examined through their effects on the mechanism of carcinogenesis. The cancer preventive effects of probiotics were investigated through their immunomodulation. In addition, the cancer preventive potential of food was explored with bifidobacteria-fermented soy milk as an example.

The effects of intestinal microflora on carcinogen-induced aberrant crypt foci have been described. To shed light on the association of intestinal microflora with the development of colon cancer, we studied the modifying effects of intestinal microflora on the occurrence aberrant crypt foci in the colon after treatment with 1,2-dimethylhydrazine in germ-free (GF), gnotobiotic (GB), and conventional (Cvd) rats. Rats were killed 11 or 34 weeks after the last DMH injections and aberrant crypt foci were counted. The total number of foci, the number of foci with 4 or more crypts, and the mean number of aberrant crypts per focus (crypt multiplicity) in GB rats killed in week 34 was 168%, 442%, and 138% of those in GF rats, respectively (by Student’s t test; all, \( P < 0.001 \)). These values for Cvd rats were 42% (\( P < 0.001 \)), 147% (\( P = 0.25 \)), and 159% (\( P < 0.001 \)) of those in GF rats, respectively. In the second part of this study, the effect of colonization of *Bifidobacterium breve* on the crypts was examined in GB rats. The number of foci with 4 or more crypts and the crypt multiplicity in GB rats infected with *B. breve* at week 11 was significantly lower than those of GB rats without *B. breve* (\( P < 0.01 \), and \( P < 0.05 \), respectively). The difference in the number of foci with 4 or more crypt was not statistically significant at week 34. These findings suggest that some intestinal bacteria might act as promoters and others might act as anti-promoters in colon carcinogenesis.

Intestinal microflora is necessary for the spontaneous development of intestinal adenocarcinoma in T-cell receptor β chain (TCR/β) and p53 double-knockout (TCR \( \beta^{-/-} \) p53\(^{-/-} \)). Next, whether intestinal microflora is needed for the development of adenocarcinoma in the colon of TCR \( \beta^{-/-} \) p53\(^{-/-} \) mouse was checked. TCR/β is a major component of the T-cell antigen receptor, and in TCR β-gene-deficient (TCR \( \beta^{-/-} \)) mice, inflammation of the intestinal tract develops. The p53 gene is a tumor suppressor gene that frequently is found to be mutated in subjects with nonhereditary malignant tumors. In addition, mutation of the p53 gene or overexpression of p53 protein is a common early event in human ulcerative colitis-associated neoplasia. p53-Gene-deficient (p53\(^{-/-} \)) mice have a high incidence of spontaneous malignant lymphomas and angiosarcomas, more evidence of a role of p53 in tumor suppression. Recent studies have shown that colitis does not develop in animal models of inflammatory bowel disease (IBD) such as IL-2\(^{-/-} \) mice, IL-10\(^{-/-} \) mice, and TCR\( \alpha^{-/-} \) mice in a germ-free environment, indicating an important role of enteric bacteria in the pathogenesis of colitis. Recently, TCR \( \beta^{-/-} \) p53\(^{-/-} \) mouse has been established; it has high incidence of spontaneous colorectal adenocarcinoma early in life. For investigation of the effects of intestinal microflora on colon cancer in such double-mutant mice, germ-free TCR \( \beta^{-/-} \) p53\(^{-/-} \) mice were produced. At 7 weeks of age, the animals were divided into two groups, each with 10 rats, and one of these groups was conventionalized. When the mice were 4 months old, they were killed and their colons were examined histopathologically for adenocarcinoma. The germ-free mice had no colonic adenocarcinomas, but in seven of the conventionalized mice, adenocarcinomas of the ileocecum and cecum were detected. These results indicate the usefulness of the TCR \( \beta^{-/-} \) p53\(^{-/-} \) mouse in a model of colon cancer in which colon adenocarcinoma occurs spontaneously early in life, and suggest that intestinal microflora are important in the development of adenocarcinoma of the colon in this animal model.

*Bifidobacterium*-fermented soy milk (FSM) inhibits rat mammary gland carcinogenesis caused by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). High levels of consumption of soybean and soybean-related products is hypothesized to help protect against breast cancer. Soybean is a rich source of genistein, a putative cancer chemopreventive agent. FSM fermented with the *Bifidobacterium breve* strain Yakult contains larger amounts of the isoflavone aglycones such as genistein and daidzein than unfermented soy milk. The effects of FSM and a mixture of isoflavones (genistein and daidzein, 4:1) on mammary carcinogenesis caused by PhIP in rats were examined. Starting when female Sprague-Dawley rats were 7 weeks old, they were given PhIP at a dose of 85 mg/kg body wt by intragastric administration four times a week for 2 weeks. They were fed a control high-fat basal feed or an experimental high-fat feed containing 10% FSM, or else a 0.02% or 0.04%...
isoﬂavone mixture, during and after carcinogen exposure. The incidence (percentage of rats with tumors) of mammary gland tumors was 71% in the control diet group, 51% in the FSM group and 68, and 61% in the groups treated with the isoﬂavone mixture at 0.02% and 0.04%, respectively. The mean +/− SD number of tumors per rat was 1.2+/−0.2 with 10% FSM, 2.2+/−0.4 with the 0.02% isoﬂavone mixture, and 1.5+/−0.3 with the 0.04% isoﬂavone mixture. These numbers are smaller than the control value of 2.6+/−0.5. Furthermore, feeding of FSM or the isoﬂavone mixture at either dose reduced the mean size of the tumors. The amount of isoﬂavones in 10% FSM is approximately equivalent to that in the 0.02% isoﬂavone mixture, so the chemopreventive activity of FSM could be partly attributed to the presence of isoﬂavones, such as genistein and daidzein. Contributions by the probiotic eﬀect of B. breve to the chemopreventive activity of FSM, however, warrants further study.

Analysis of intestine-modulating foods

The intestine has a variety of functions essential to the maintenance of human health (Fig. 3). Digestion of food and absorption of nutrients are carried out by the intestinal epithelium, which also acts as a barrier, protecting the body from invasion by harmful substances and microorganisms. The epithelium recognizes signals derived from food and transfers the signals into the body. Beneath the epithelial cell monolayer, there are varieties of cells that interact with each other, producing various bioactive substances. Modulation of these functions by food components would therefore affect the physiological state.

Intestinal functions can be modulated by various factors that include intestinal microﬂora, food factors, and internal factors such as cytokines.

Intestinal conditions can be improved by regulation of the intestinal microﬂora. Probiotics and prebiotics are receiving attention as candidate regulators. The term “probiotics” is deﬁned as “living microorganisms having beneﬁcial properties for the host including improving the balance of host intestinal microﬂora”. Bifidobacterium species and lactic bacteria are examples. The term “prebiotics” refers to “nondigestible food substances that pass through the small intestine and are fermented by endogenous microﬂora”. Dietary ﬁbers and certain oligosaccharides are examples. The use of prebiotics and probiotics strengthen the indigenous gut microﬂora to inhibit the growth and colonization of potentially pathogenic bacteria. Some gut bacteria (e.g., lactobacilli) also improve intestinal immune functions. Increased secretion of certain cytokines from the intestinal epithelial cells in the presence of gut bacteria has been reported. Prebiotics are fermented by gut bacteria, producing short-chain fatty acids, including propionic and butyric acids, which provide energy for intestinal epithelial cells. However, these acids also aﬀect the growth and diﬀerentiation of intestinal cells, thereby modulating intestinal functions. The role of gut microﬂora in protecting the digestive tract, as well as in modulating the immune system, is one area of active research in this ﬁeld of functional foods.

Intestinal functioning is modulated not only by gut microﬂora but also by food factors. Various food substances that aﬀect the activity of digestive enzymes (e.g., α-glycosidases and peptidases), nutrient transporters (e.g., transporters of glucose, amino acids, peptided, vitamins), and tight junctions have been reported. For example, green tea polyphenols such as epicatechin gallate inhibit the sodium-depen dent glucose transporter SGLT1 speciﬁcally. Tea catechins inhibit intestinal α-glycosidases. The increase in the blood glucose level after a meal may therefore be suppressed by the drinking of green tea. Recent studies suggest that the activity of intestinal P-glycoprotein, an ATP-dependent active eﬄux transporter for hydrophobic xenobiotics and drugs, can be aﬀected by certain food factors. Excretion of xenobiotics or drug absorption might be controlled to some extent by food. An increase in tight junction permeability caused by food factors would increase the absorption of water-soluble compounds of small molecular weight such as mineral ions, vitamins and bioactive oligopeptides. Absorption of nutrients would be more eﬃcient with these food factors. Alteration by food factors of tight junction permeability may also be related to activation of the gut immune system and to the internalization of pathogenic bacteria by enterocytes.

Food substances are often chemically modiﬁed by intestinal epithelial cells. For example, curcumin, an antioxidative substance in turmeric, is converted to tetrahydrocurcumin, which has more antioxidative
activity than curcumin, during the process of absorption from the intestine. Compounds in the intestinal epithelial cells are detoxified by enzymes, including glutathione S-transferase (GST). Modulation of the activities of these enzymes by food substances has been reported. Therefore the intestinal absorption, excretion, and metabolism of nutrients, functional substances, and xenobiotics may be, at least partly, regulated by food factors.

Intestinal functions are regulated by internal factors such as cytokines and growth factors. Tight junction permeability is regulated by various cytokines, with permeability being increased by IFN-γ and TNF-α, and permeability being reduced by transforming growth factor-β (TGF-β). Transporters also are regulated by cytokines. We have recently observed that expression of the intestinal taurine transporter is up-regulated by TNF-α.

Intestinal epithelial cells themselves can produce a variety of bioactive substances, including cytokines, growth factors, and hormones. The local concentration of cytokines, such as TGF-β, IL-8 and monocyte chemotactic protein-1 (MCP-1), in the intestinal epithelium would be increased if intestinal epithelial cells are stimulated with certain food substances. These cytokines modulate growth and differentiation and the function of cells beneath the epithelial cell layer. Nagafuchi et al. have recently reported that dietary nucleotides increase the production of IL-7 by intestinal epithelial cells, thereby modulating the growth and differentiation of intraepithelial T-lymphocytes. It is likely that the intestinal epithelium is not a simple gate for nutrients and food factors but also acts as a “converter” of food factors or food signals. The intestinal epithelium must be important in the phenomenon of food functionality. Development of new experimental methods, including the coculture technique, should make clear the detailed mechanisms of interactions between intestinal cells and food substances.

As of May 2002, 298 items of FOSHU, “Foods for Specified Health Use”, have been approved in Japan, about two-thirds of which are based on the mechanisms controlling intestinal events such as described here. Probiotics and prebiotics have been designed to improve the gastrointestinal condition. Increases in mineral absorption, retardation of triglyceride synthesis, and inhibition or retardation of glucose absorption in the intestine are the main mechanisms of those FOSHU products. The modulation of intestinal function could be a practical and promising target for the development of new functional foods.

Since the first cultivation of *Helicobacter pylori* in 1982, evidence of a relationship between *H. pylori* and gastroduodenal diseases such as chronic inflammation and peptic ulcer has become clear. This bacterium also is implicated in gastric cancer. Because the infection rate of *H. pylori* is high, about 50% in the Japanese population, it is impossible to eradicate *H. pylori* in all infected subjects. Eradication therapy using antibiotics is not always successful, and may be associated with side effects. Studies aimed at developing an anti-*H. pylori* lactic acid bacterium were started for these reasons.

The properties needed for anti-*H. pylori* effects (resistance to artificial gastric juice, proliferation under acid conditions, adherence to cultured gastric epithelial cells, suppression of *H. pylori* during co-fermentation) of 203 *Lactobacillus* strains were examined in vitro. *Lactobacillus* gasseri OLL2716 (LG21), *Lactobacillus gasseri* No. 6, and *Lactobacillus salivarius* WB1004 were selected as lactic acid bacteria with potentially anti-*H. pylori* properties. After in vitro experiments, the effects of administration of the selected *Lactobacillus* strains on mice infected with *H. pylori* were examined. *H. pylori* cells (10⁶ cfu) were administered orally to germ-free mice once daily for 3 days to bring about infection. Six weeks after the administration of *H. pylori* ended, *Lactobacillus* strains (10⁶ cfu) were administered orally once weekly for 8 weeks. One week after the dosing of the *Lactobacillus* strains ended, the number of *H. pylori* cells and serum anti-*H. pylori* IgG levels were investigated by quantitative culture and ELISA respectively. No *H. pylori* cells were detected from mice given LG21 or *L. salivarius* WB1004 cells, and the anti-*H. pylori* IgG level of mice given LG21 cells was the lowest. Therefore, LG21 was the most suitable strain for use as a probiotic against *H. pylori*.

To examine the efficacy of strain LG21 cells against *H. pylori* infection in humans, a clinical study was done. Thirty-one healthy volunteers infected with *H. pylori* ingested 90 g of yogurt twice a day for 8 weeks. From 1 week after the end of this 8-week period, the volunteers ingested 90 g of yogurt containing 10⁶ cfu of LG21 cells twice daily for 8 weeks. The urea breath test and assays of serum pepsinogen I and II were done to measure the number of *H. pylori* cells and to evaluate the degree of mucosal inflammation, respectively. Measurements were taken before the intake of the ordinary yogurt, 1 week after the end of the intake of ordinary yogurt, and 1 week after the end of the intake of yogurt containing LG21 cells. The results of the urea breath test did not significantly change after the intake of ordinary yogurt, but did significantly decrease following intake of the yogurt containing LG21 cells (Fig. 4). The pepsinogen I/II ratio did not significantly change after the intake of ordinary yogurt, but did sig-

**Development of intestine modulating food—anti-*Helicobacter pylori* lactic acid bacterium**
significantly increase following the intake of yogurt containing strain LG21 cells (Fig. 5).

Next, the effects of long-term administration of yogurt containing LG21 cells were examined. Thirty-one healthy volunteers infected with *H. pylori* ingested 120 g of yogurt containing $10^9$ cfu of LG21 cells daily for 24 weeks. The urea breath test and assays of serum pepsinogen I and II were done before and 1 week after the end of the 24-week period of intake of yogurt containing LG21. The $\Delta^{13}$C value significantly decreased (mean ± S.D., 22.3 ± 15.8 vs. 16.0 ± 10.5; $p < 0.01$), and the serum pepsinogen I/II ratio had significantly increased (mean ± S.D., 2.69 ± 1.12 vs. 2.95 ± 1.32; $p < 0.05$) following intake of yogurt containing LG21 cells. No volunteers reported adverse health effects during the test period when asked.

These results suggest that the intake of yogurt containing LG21 cells decreases the number of *H. pylori* and also reduces the degree of mucosal inflammation in the stomach of humans infected with *H. pylori*. LG21 thus can affect *H. pylori* in both in vitro and animal studies. In the clinical studies, the intake of yogurt containing LG21 cells decreases the number of *H. pylori* cells and relieves the severity of mucosal inflammation in the stomach of humans infected with *H. pylori*. The density of *H. pylori* colonization in the stomach affects the progression after infection with this bacterium. There is significant correlation between *H. pylori* cell density and gastric inflammation and duodenal ulceration. No duodenal ulceration was found in subjects with antral *H. pylori* densities of less than $10^5$ cfu/g of tissue protein. Because the intake of yogurt containing LG21 cells suppresses *H. pylori* in addition to reducing inflammation in the stomach, such cells may also reduce the risk of *H. pylori*-induced gastrointestinal diseases.

**Concluding remarks**

The population of Japan is aging, and diseases related to lifestyle are on the increase over recent years. Society will have to carry the burden of escalating medical costs associated with treating more people with such diseases. For the prevention of these diseases, a focused approach to the health aspects of functional foods should be of benefit. From the scientific viewpoint, an understanding of the health-promoting effects of foods is of interest to scientists whose task it is to elucidate the nutritional benefits of foods. The concept of functional food originated from a study group for the systemic analysis and development of food functions. The science of functional food has advanced since that time with a large number of functional food components, such as food-protein-derived peptides with physiological activities, probiotics, prebiotics, and antioxidants having been developed.

The Japanese government has been active in promoting the role of food in maintaining good health and in disease prevention. The government has created a special regulatory category for functional food and has established the “Food for Specified Health Use (FOSHU)” approval system. FOSHU foods are foods with specific effects contributing to the maintenance of health. For their approval, experimental data that show the scientific relationship between a food’s components and its health-related benefits are needed.

FOSHU foods contain food components, that are not regarded as drugs but do possess some physiologically beneficial functions. The assessment of FOSHU foods for approval for human consumption is not as severe as that for pharmaceutical drugs. For
Table 3. FOSHU Products (April 2001)

<table>
<thead>
<tr>
<th>Health claim</th>
<th>Functional component</th>
<th>Number of food products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helps maintains good gut condition</td>
<td>Oligosaccharide</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>Lactic acid bacteria</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dietary fiber</td>
<td></td>
</tr>
<tr>
<td>Helpful for people concerned about</td>
<td>Soybean protein</td>
<td>18</td>
</tr>
<tr>
<td>blood cholesterol levels</td>
<td>Chitosan, etc.</td>
<td></td>
</tr>
<tr>
<td>Helps reduce neutral fats</td>
<td>Diacylglycerol, etc.</td>
<td>10</td>
</tr>
<tr>
<td>Suitable for those with mild</td>
<td>Casein decapeptide</td>
<td>20</td>
</tr>
<tr>
<td>hypertension</td>
<td>Lactoprotein, etc.</td>
<td></td>
</tr>
<tr>
<td>Helps reduce blood glucose</td>
<td>Indigestible dextrin</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Wheat albumin, etc.</td>
<td></td>
</tr>
<tr>
<td>Improves mineral absorption</td>
<td>Casein phosphopeptide</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Fructoligosaccharides, etc.</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>256</td>
</tr>
</tbody>
</table>

that reason, FOSHU foods cannot be marketed with the claim that they have disease-curing properties. Instead, such foods carry claims that merely suggest health-promoting or condition-preventing effects. For example, a claim might be that a food (1) helps to maintain good gastrointestinal function, (2) is helpful for those who are concerned about high blood cholesterol levels, (3) helps to reduce neutral fat, (4) is suitable for people who suffer from mild hypertension, (5) helps to reduce blood glucose, (6) improves mineral absorption, and so on. Thirteen products were approved as FOSHU foods in 1993–1994, and the total number of approvals reached 260 by May 2001. Table 3 summarized the FOSHU foods approved to date.

Gastrointestinal health is the most important category of FOSHU-approved products, accounting for 60% of such approvals as of the end of October 2001. Categories of FOSHU-approved products include those influencing hyperlipidemia, mineral absorption, hypertension, dental caries, and hyperglycemia, to name a few. There are certain components of FOSHU foods that are more common than others. For example, numerous products use probiotic bacteria, oligosaccharides, soy protein, or indigestible dextrin.

In April 2001, the government established a new system regulating functional foods. In addition to the FOSHU approval system, the new category “food with nutrient function claims” was created. FOSHU foods and foods with nutrient function claims were included in a new category of “food with health claims” (Fig. 6). It should be noted that the FOSHU approval system has been modified. The new approval system states that the physiological functions and safety of foods should be assessed in humans. Foods with nutrient claims have high levels of vitamins and minerals, such as vitamins A, B, B2, B6, B12, C, D, E, folic acid, niacin, pantothenic acid, and biotin as well as calcium and iron. Health claims can be made without submitting the foods to the nutrient claim approval system if the foods contain sufficient amounts of nutritional functional food components as judged from standards set by the government.

Many functional foods have been developed in Japan. For example, Japanese scientists have discovered many types of physiological components: antioxidative, cancer-preventive, anti-hypertensive, and immuno modulative. These components seem to be effective for lifestyle-related diseases and, in fact, there is much experimental evidence available of their physiological functions. These functional components will become more important in the prevention of diseases in the future. However, most of them have not been approved as FOSHU foods. These new kinds of functional foods need to be submitted to government assessment.

A large number of functional foods are being developed in other countries, where their physiological functions are being investigated. State-of-the-art functional food science in Europe has been reviewed. The review suggested that functional foods for obesity, diabetes, undernutrition, cancer, cardiovascular disease, stimulation of the gastrointestinal immune system, and activation of intestinal microflora are needed by many people globally. Behavioral and psychological effects of foods have also been described. The materials described as important functional foods in that review are similar to those used in Japan. However, they seem to focus more on immunomodulating food components such as probiotics and prebiotics, and antioxidative materials such as vitamins and flavonoids, rather than an other specific components. Both FOSHU from Japan and functional foods developed in other countries should contribute to the health and welfare of all humankind.

Acknowledgments

This is an overview compiled from the papers presented at the IUFoST-Japan “Functional Food Science” Symposium (Tokyo, November 14, 2001), and we express our sincere thanks to various scientific bodies and their supporters. These are the Japan Society for Bioscience, Biotechnology, and Agrochemistry, the Japanese Society for Food Science and Technology, the Japanese Society of Nutritional and
Food Science, the Japan Association for Animal Cell Technology, the Japan Society for Food Factors, and the Institute of Food Technologists-Japan, as well as ILSI Japan. We are also grateful to the IUFoST-Japan advisors: Dr. H. Mitsuda, Emeritus Professor of Kyoto University, Dr. M. Fujimaki, Emeritus Professor of the University of Tokyo and Ochanomizu University, Professor D. Kanamori, Emeritus Professor of Kyoto Prefectural University and Mukogawa Women’s University, and Professor K. Yasumoto, Sugiyama Gakuen Women’s University, and HLSI-Japan Chairman, Professor S. Kimura, Showa Women’s University, for a vast amount of helpful advice. Our special thanks are offered to Dr. A. Yamanoi, Ajinomoto Co., Ltd., Dr. T. Hirahara, Calpis Co., Ltd., Professor T. Osawa, Nagoya University, Professor H. Ohigashi, Kyoto University, Professor T. Ogawa, Kyoto University, and Dr. K. Shinohara, National Food Research Institute, for interesting discussions during the symposium.

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