

Review Article

A model for the role of the proline-linked pentose-phosphate pathway in phenolic phytochemical biosynthesis and mechanism of action for human health and environmental applications*

Kalidas Shetty PhD¹ and Mark Wahlqvist AO, MD, FRACP²¹Laboratory of Food Biotechnology, Department of Food Science, Chenoweth Laboratory, University of Massachusetts, Amherst, MA 01003, USA²Asia Pacific Health & Nutrition Centre, Monash Asia Institute, Monash University, Melbourne, Australia**This manuscript is dedicated to Huang Soo Sien for her support, friendship, dignity and wisdom.*

The combination of immunodeficiency, inflammatory process and nutritional status that is characteristic of infective and food-borne illness is more evident in chronic diet- and environment-influenced chronic diseases such as diabetes, obesity, cardiovascular disease, cancer, arthritis and neuro-degeneration diseases. These chronic diseases tend to be oxidation-linked and may manifest in communities around the world, irrespective of income. In addressing the challenges of the above diseases, a significant role for dietary phytochemicals is emerging. Phytochemicals are required from a spectrum of food for at least their antioxidant role, if not for other properties, to protect tissues from activities that manifest themselves into what we call chronic disease. Among the diverse groups of phytochemicals, phenolic antioxidants and antimicrobials from food plants are being targeted for designed dietary intervention to manage major oxidation-linked diseases such as diabetes, cardiovascular diseases, arthritis, cognition diseases and cancer. Foods containing phenolic phytochemicals are also being targeted to manage bacterial infections associated with chronic diseases such as peptic ulcer, urinary tract infections, dental caries and food-borne bacterial infections. Plants produce phenolic metabolites as a part of growth, developmental and stress adaptation response. These stress and developmental responses are being harnessed to design consistent phytochemical profiles for safety and clinical relevancy using novel tissue culture and bioprocessing technologies. The biochemical strategy for harnessing phenolic phytochemicals for human health and wellness is based on the hypothesis that phenolic metabolites in plants are efficiently produced through an alternative mode of metabolism linking proline synthesis with pentose-phosphate pathway. In this model, stress-induced proline biosynthesis is coupled to pentose-phosphate pathway, driving the synthesis of NADPH₂ and sugar phosphates for anabolic pathways, including phenolic and antioxidant response pathways, while simultaneously providing reducing equivalents needed for mitochondrial oxidative phosphorylation in the form of proline as an alternative to NADH from Krebs/TCA cycle. Based on this model, tissue culture techniques and elicitation concepts have been used to stimulate phenolic metabolites with an antioxidant response in germinating seeds, sprouts and clonal lines of dietary plants. From our initial investigations, a model has been proposed in which the proline-linked pentose-phosphate pathway is suggested to be critical for modulating protective antioxidant response pathways in diverse biological systems, including biochemical and cellular pathways important for human health. The proposed proline-linked pentose-phosphate pathway model provides a mechanism for understanding the mode of action of phenolic phytochemicals in modulating antioxidant pathways and provides avenues by which dietary approaches may manage oxidation-linked chronic and infectious diseases. The model also has implications for the development of antimicrobial phenolic phytochemicals against bacterial pathogens in an era of increasing antibiotic resistance. Further, this model also has relevance for improving fungal and yeast-based food bioprocessing for designing functional foods and for environmental bioremediation using plant and microbial systems, as well as for improving agricultural and food systems in harsh environments.

Key Words: antioxidant response, antimicrobials, chronic disease, environmental applications, human health, oxidation-linked disease, oxidative phosphorylation, phenolic antioxidants, phytochemicals, proline-linked pentose phosphate pathway.

Introduction to food and nutrition issues and relevance of phenolic antioxidants

Increasing efforts are being made world wide to address both persistent nutritional deprivation in economically disadvantaged communities and the increase of chronic oxidation-linked diseases (i.e., abdominal obesity, diabetes, cardiovascular disease (CVD), certain cancers, osteoporosis, arthritis and inflammatory diseases) in all communities that have reached caloric and protein sufficiency. The

combination of immunodeficiency, inflammatory process and nutritional status that is characteristic of infective and food-borne illness is more evident in chronic diet- and environment-influenced diseases. Therefore such diseases

Correspondence address: Prof Kalidas Shetty, Dept Food Science, Chenoweth Lab., Uni. Massachusetts, Amherst, MA 1003, USA. Tel: +1-413-545-1022; Fax: + +1-413-545-1262; kalidas@foodsci.umass.edu

Accepted 5 June 2003

in fact may be considered as 'eco-diseases' with environmental and behavioural contributors such as physical inactivity as opposed to nutritionally-dependent 'econutritional diseases'.¹ These chronic diseases are oxidation-linked and manifest in communities around the world, irrespective of income or age. In addressing the above challenges, a significant role for phytochemicals in the diet is emerging. Dietary phytochemicals are required for, at least, their antioxidant role, if not other properties to protect tissues from chronic disease.²

Food plants are excellent sources of phenolic phytochemicals, especially as antioxidants. While phenolic antioxidants from dietary sources have a history of use in food preservation, many increasingly have therapeutic and disease prevention applications.³⁻⁶ Therefore, understanding the nutritional and therapeutic role of dietary phytochemicals and particularly phenolic antioxidants is an important scientific agenda for Food Science and Nutrition, now and well into the foreseeable future.⁷ This role is becoming very significant at a time when the importance of phytochemicals in the prevention of oxidation-linked chronic diseases is gaining rapid recognition globally. Disease prevention and management through the diet can be considered an effective tool to improve health and/or reduce the increasing health-care costs for these oxidation-linked chronic diseases, especially in low-income countries.

Phenolic phytochemicals have been associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals.⁸⁻¹¹ Recent studies have indicated a role for phenolics from food plants in human health and, in particular cancer.^{10,12} Phenolic phytochemicals (i.e. phenylpropanoids) serve as effective antioxidants due to their ability to donate hydrogens from hydroxyl groups positioned along the aromatic ring to terminate free radical oxidation of lipids and other biomolecules.¹³ Phenolic antioxidants, therefore, short-circuit a destructive chain reaction that ultimately degrades cellular membranes.

Plant phenolics are an important sub-group of secondary metabolites, which have diverse functional and medicinal applications. Examples of phenolics that are used as antioxidant and anti-inflammatory compounds are curcumin from *Curcuma longa*,¹⁴⁻¹⁶ *Curcuma mangga*,¹⁷ and *Zingiber cassumunar*,¹⁸ and rosmarinic acid from *Rosmarinus officinalis*.^{6,19} Examples of phenolics with cancer chemopreventive potential are curcumin from *Curcuma longa*,^{4,20-23} isoflavonoids from *Glycine max*²⁴⁻²⁶ and galanigin from *Origanum vulgare*.²⁷ Other examples of plant phenolics with medicinal uses include lithospermic acid from *Lithospermum* sp. as anti-gonodotropic agent,²⁸ salvianolic acid from *Salvia miltiorrhiza* as an antiulcer agent,²⁹ proanthocyanidins from cranberry to combat urinary tract infections,^{30,31} thymol from *Thymus vulgaris* for anti-caries,³² and anethole from *Pimpinella anisum* as an antifungal agent.³³

Important plant diphenyl metabolites targeted for enhanced production against oxidation-linked disease are rosmarinic acid, resveratrol, ellagic acid and curcumin. Other phenolic phytochemicals also targeted are flavonoids, quercetin, myricetin, scopoletin and isoflavonoids. Among simple phenolics, there is major interest

in the over-expression of L-tyrosine and L-DOPA from legumes in a high-phenolic antioxidant background.^{34,35} Rosmarinic acid has been targeted from clonal herbs³ for its anti-inflammatory and antioxidant properties.^{19,36,37} Resveratrol has shown antioxidant and cancer chemopreventive properties^{38,39} and its over-production has been targeted from several fruits using solid-state bioprocessing.^{40,41} Ellagic acid has been targeted for antioxidant and cancer chemopreventive properties^{42,43} and has been similarly targeted via solid-state bioprocessing from fruits and fruit processing byproducts.⁴⁰ As extensive studies have shown cancer chemopreventive and antioxidant properties for *Curcuma longa* and its major active compound, curcumin,^{15,20,44} and its elicitor and physical stress-mediated over expression of curcumin is being investigated.

The emergence of dietary and medicinal applications for phenolic phytochemicals, harnessing their antioxidant and antimicrobial properties, in human health and wellness is not altogether surprising. As stress damage on the cellular level appears similar among eukaryotes, it is logical to suspect that there may be similarities in the mechanism for cellular stress mediation between eukaryotic species. Plant adaptation to biotic and abiotic stress involves the stimulation of protective secondary metabolite pathways⁴⁵⁻⁴⁷ that results in the biosynthesis of phenolic antioxidants. Studies indicate that plants exposed to ozone responded with increased transcript levels of enzymes in the phenylpropanoid and lignin pathways.⁴⁸ Increase in plant thermo-tolerance is related to the accumulation of phenolic metabolites and heat shock proteins that act as chaperones during hyperthermia.⁴⁹ Phenolics and specific phenolic-like salicylic acid levels increase in response to infection, acting as defence compounds or to serve as precursors for the synthesis of lignin, suberin and other polyphenolic barriers.⁵⁰ Antimicrobial phenolics called phytoalexins are synthesized around the site of infection during pathogen attack and, along with other simple phenolic metabolites, are believed to be part of a signalling process that results in systemic acquired resistance.⁴⁵⁻⁴⁷ Many phenylpropanoid compounds such as flavonoids, isoflavonoids, anthocyanins and polyphenols are induced in response to wounding,⁵¹ nutritional stress,⁵² cold stress⁵³ and high-visible light.⁵⁴ UV irradiation induces light-absorbing flavonoids and sinapate esters in *Arabidopsis* to block radiation and protect DNA from dimerization and/or cleavage.⁵⁵ In general, the initiation of the stress response arises from certain changes in the intracellular medium⁵⁶ that transmit the stress-induced signal to cellular modulating systems and results in changes in cytosolic calcium levels, proton potential as a long-distance signal,⁵⁷ and low-molecular weight proteins.⁵⁸ Stress can also initiate free radical generating processes and shift the cellular equilibrium towards lipid peroxidation.⁵⁹ It is believed that the shift in prooxidant-antioxidant equilibrium is a primary non-specific event in the development of the general stress response.⁶⁰ Therefore, protective phenolic antioxidants involved in such secondary metabolite-linked stress responses in food plant species can be targeted as a source of therapeutic and disease-preventing functional ingredients. This is especially applicable to: 1) oxidation

disease-linked diet problems (high glycaemic index and saturated fats) and 2) environment-influenced (physical, chemical and biological) chronic disease problems.

Role of proline in plants

Proline biosynthesis is a common stress response in plants, especially to water and salinity stress.⁶¹⁻⁶⁵ This amino acid accumulates in saline-tolerant halophilic plants,⁶⁶ under water stress,⁶⁷ in desiccating pollen,⁶⁸ plant cell culture under water stress,⁶⁹ salt stress,^{70,71} during senescence,⁷² in response to abscisic acid,⁷³ proline analogue,⁷⁴ dehydration,⁷⁵ freezing tolerance,⁷⁶ general osmotic tolerance over-expressing proline synthesis pathway⁷⁷ and frost tolerance.⁷⁸ Proline is suggested to protect membranes and proteins from the various imposed stress conditions mentioned above.^{79,80} Proline may also act as an antioxidant through hydroxyl radical scavenging activity.⁸¹ In microorganisms, especially bacteria, proline over-expression can increase osmo-tolerance.⁸²⁻⁸⁴

Proline is also suggested to have roles in plants in addition to stress tolerance. Proline synthesis is known to be stimulated during senescence.⁷² Further, it has been implicated in energy transfer and regulation of purine synthesis in nitrogen fixing root nodules in soybean.^{85,86} It has been linked to development-linked rhizogenesis associated with drought⁸⁷ and thermogenic response in inflorescence of Voodoo lily.⁸⁸ It has also been associated with non-stress related developmental conditions. In the fava bean, the ratio of free and bound proline content was very high during early pod development and decreased during late stages at maturity.⁸⁹ The very high proline levels were not a consequence of water stress. It was suggested that free proline has a characteristic function in early development.⁸⁹ Previous studies in soybean⁹⁰ and peanut⁹¹ have indicated that accumulation of proline was not associated with water stress. A function of proline in formation of flowers has also been suggested.⁹²

Proline in plant tissue culture

Proline alone or in combination with other amino acids is known to stimulate somatic embryogenesis in plants. Proline in combination with serine, stimulated somatic embryogenesis in orchard grass.⁹³ Proline alone stimulated *in vitro* somatic embryogenesis in maize⁹⁴ and carrot.⁹⁵ Proline and glutamine stimulated induction of embryogenic callus in a grass species *Agrostis alba*.⁹⁶ Further, the proline analogue, thioproline was used to screen highly embryogenic cell lines from single seed origin in a heterogeneous genetic background of *Agrostis alba*.⁹⁷ Proline has been used to stimulate cytokinin-induced *in vitro* shoot organogenesis in melon⁹⁸ and cucumber,⁹⁹ as well as auxin-induced somatic embryogenesis on alfalfa.¹⁰⁰ Proline, proline analogue and proline precursor, ornithine were effectively used to stimulate cytokinin-induced shoot organogenesis in melon.¹⁰¹ *In vitro* shoot organogenesis was also stimulated using proline and glutamic acid-enriched fish protein hydrolysates in combination with proline analogues.¹⁰² It was clear from the melon study that the extent of shoot organogenesis was correlated to increased proline content

in such differentiated tissues.^{101,102}

An interesting alternative role for proline in stress-induced phenolic synthesis has been proposed.³ This concept is based on the original model on the role of proline and pyrroline-5-carboxylate (P5C) in regulating redox and hydride ion-mediated stimulation of pentose-phosphate pathway, which in turn modulates purine metabolism in animal cells.¹⁰³ In this model,¹⁰³ stimulation of proline synthesis regulates the ratios of NADP⁺/NADPH₂ and sugar phosphates (that are required for purine metabolism) through the stimulation of the NADPH₂-producing committed steps of the pentose-phosphate pathway. Further, it was proposed that rapid catabolism of proline during recovery from stress may provide redox equivalents for mitochondrial oxidative phosphorylation and therefore it is an alternative route to ATP synthesis.³ Extending from the model proposed in animal systems by Phang¹⁰³ the role of proline-linked pentose phosphate pathway in stimulating phenolic metabolites in plants was proposed by Shetty³ (Fig. 1).

Proline-linked phenolic synthesis in herb clonal shoot culture systems

The hypothesis that synthesis of plant phenolic metabolites is linked to the proline-linked pentose-phosphate pathway³ (Fig. 1) was developed based on the role of the proline-linked pentose-phosphate pathway in regulation of purine metabolism in mammalian systems.¹⁰³ Proline is synthesized by a series of reduction reactions from glutamate. In this sequence, P5C and proline, known to be metabolic regulators, function as a redox couple.^{103,104} During respiration, oxidation reactions produce hydride ions, which augment reduction of P5C to proline in the cytosol. Proline can then enter mitochondria through proline dehydrogenase¹⁰⁵ and support oxidative phosphorylation (alternative to NADH from Krebs/TCA cycle). This is important because shunting the TCA cycle towards proline synthesis likely deregulates normal NADH synthesis. The reduction of P5C in the cytosol provides NADP⁺, which is the co-factor for glucose-6-phosphate dehydrogenase (G6PDH), an enzyme that catalyses the rate-limiting step of the pentose-phosphate pathway. Proline synthesis is therefore hypothesized and has been partly shown to both regulate and stimulate pentose-phosphate pathway activity in erythrocytes¹⁰⁶ and cultured fibroblasts¹⁰⁷ when P5C is converted to proline. This was shown to stimulate purine metabolism via ribose-5-phosphate, which affects cellular physiology and therefore function.^{103,108}

From the above insights Shetty³ proposed a model that proline-linked pentose-phosphate pathway could stimulate shikimate and phenylpropanoid pathways and hypothesized that stress-linked modulation of this pathway lead to the stimulation of phenolic phytochemicals³ (Fig. 1). Using this model, proline, proline precursors, and proline analogues were effectively utilized to stimulate total phenolic content and a specific phenolic metabolite, rosmarinic acid.^{109,110} Further, it was shown that proline, proline precursors, and proline analogues stimulated somatic embryogenesis in anise,

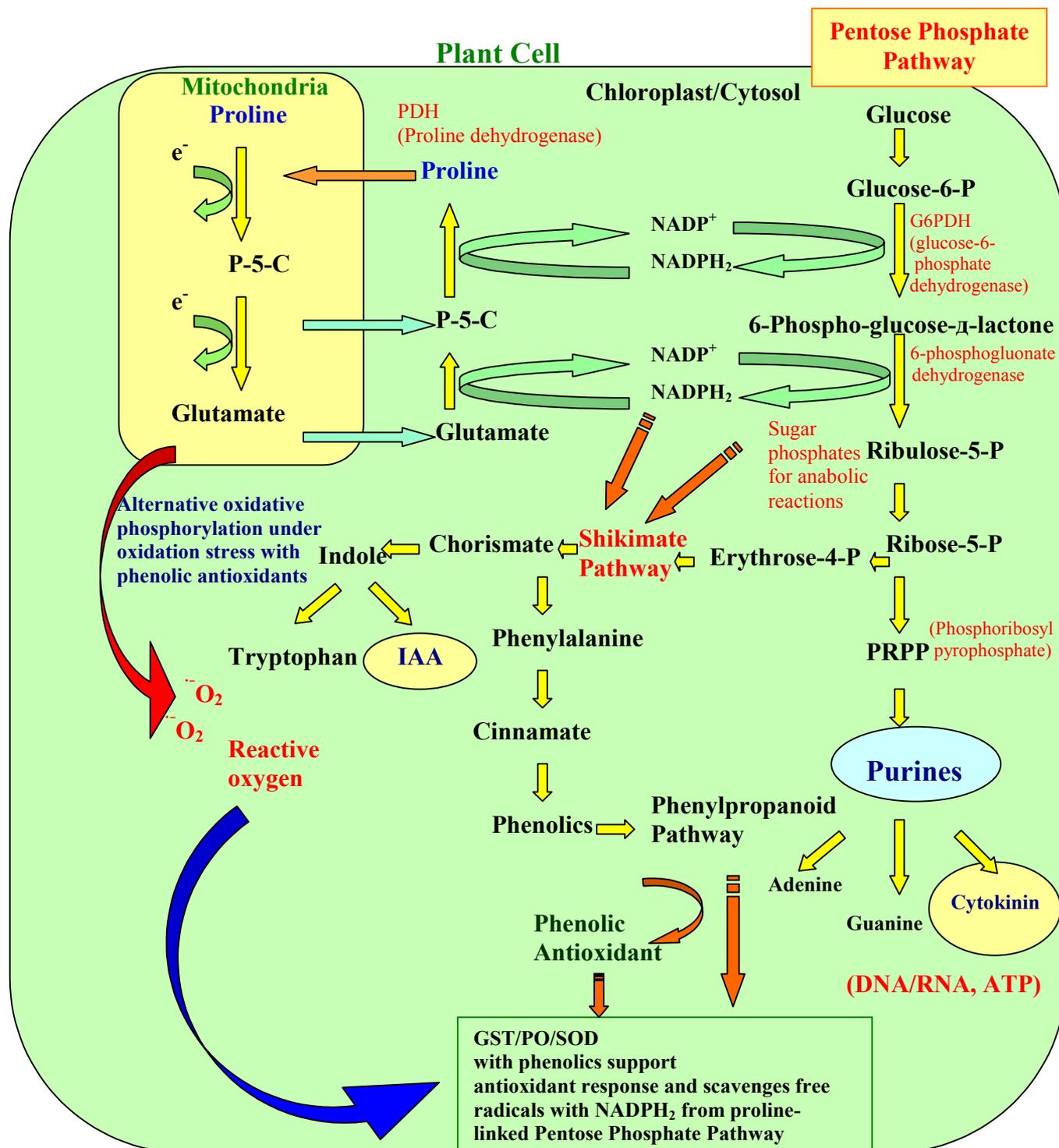


Figure 1. Original proposed model (Shetty, 1997) for the role of proline-linked pentose phosphate pathway in regulating phenolic biosynthesis. (Abbreviations: P5C;pyrroline-5-carboxylate, IAA; indole acetic acid, GST; Glutathione-s-transferase, PO;peroxidase, SOD; superoxide dismutase).

which correlated with increased phenolic content.¹¹¹ It was also established that during *Pseudomonas*-mediated stimulation of total phenolic and rosmarinic acid (RA), proline content was stimulated in oregano clonal shoot cultures.¹¹² Therefore, it was proposed that NADPH_2 demand for proline synthesis during response to microbial interaction and proline analogue treatment³ may reduce the cytosolic $\text{NADPH}_2/\text{NADP}^+$ ratio, which should activate G6PDH.^{113,114} Therefore, deregulation of the pentose-phosphate pathway by proline analogue- and microbial-induced proline synthesis may provide the excess

erythrose-4-phosphate (E4P) for shikimate and, therefore, the phenylpropanoid pathways. At the same time, proline and P5C could serve as superior reducing equivalents (RE), alternative to NADH (from Krebs/TCA cycle) to support increased oxidative phosphorylation (ATP synthesis) in the mitochondria during the stress response.^{103,104}

The proline analogue, azetidine-2-carboxylate (A2C), is an inhibitor of proline dehydrogenase.⁷⁴ It is also known to inhibit differentiation in Leydig cells of rat fetal testis, which can be overcome by exogenous proline addition.¹¹⁵ Another analogue, hydroxyproline, is a competitive

inhibitor of proline for incorporation into proteins. According to the model of Shetty,³ either analogue at low levels should deregulate proline synthesis from feedback inhibition and stimulate proline synthesis.³ This would then allow the proline-linked pentose-phosphate pathway to be activated for NADPH₂ synthesis, and concomitantly drive metabolic flux towards E4P for biosynthesis of shikimate and phenylpropanoid metabolites, including RA. Proline could also serve as a RE for ATP synthesis via mitochondrial membrane-associated proline dehydrogenase.¹⁰⁵

High RA-producing, shoot-based clonal lines originating from a single heterozygous seed among a heterogeneous bulk-seed population of lavender, spearmint and thyme have been screened and isolated based on tolerance to the proline analogue, A2C and a novel *Pseudomonas* sp. isolated from oregano.¹¹⁶⁻¹¹⁸ This strategy for screening and selection of high RA clonal lines is also based on the model that proline-linked pentose phosphate pathway is critical for driving metabolic flux (i.e E4P) towards shikimate and phenylpropanoid pathways (Fig. 1). Any clonal line with a deregulated proline synthesis pathway should have an over-expressed pentose phosphate pathway which allows excess metabolic flux to drive shikimate and phenylpropanoid pathway towards total phenolic and RA synthesis. Similarly, such proline over-expressing clonal lines should be more tolerant to proline analogue, A2C. If the metabolic flux to RA is over-expressed, it is likely to be stimulated in response to *Pseudomonas* sp. Therefore, such a clonal line is equally likely to be tolerant to *Pseudomonas* sp. Further, such a clonal line should also exhibit high proline oxidation and RA content in response to A2C and *Pseudomonas* sp. In addition, in the presence of A2C or *Pseudomonas* sp., increased activity of key enzymes G6PDH (pentose-phosphate pathway), P5C reductase (proline synthesis pathway), proline dehydrogenase (proline oxidation pathway), 3-deoxy-D-arabino heptulosonate-7-phosphate synthase (shikimate pathway), and phenylalanine ammonia-lyase (phenylpropanoid pathway) should be stimulated. The rationale for this model is based on the role of the pentose-phosphate pathway in driving ribose-5-phosphate towards purine metabolism in cancer cells,¹⁰³ differentiating animal tissues,¹¹⁵ and plant tissues.⁸⁵ The hypothesis of this model is that the same metabolic flux from over expression of proline-linked pentose-phosphate pathway regulates the inter-conversion of ribose-5-phosphate to E4P driving shikimate pathway. Shikimate pathway flux is critical for both auxin and phenylpropanoid biosynthesis, including RA. This hypothesis has been strengthened by preliminary results in which RA biosynthesis in several oregano clonal lines were significantly stimulated by exogenous addition of proline analogue (e.g A2C) and ornithine.^{109,110} The same clonal lines are also tolerant to *Pseudomonas* sp. and respond to the bacterium by increasing RA and proline biosynthesis.^{112,119} High RA-producing clonal lines selected by approaches based on this model¹¹⁶⁻¹²¹ are being targeted for preliminary characterization of the key enzymes mentioned above. The success of this strategy will lead to access of critical interlinking metabolic pathways asso-

ciated with RA biosynthesis and will allow more detailed analyses, which could lead to metabolic engineering for efficient RA biosynthesis. This strategy for investigation and stimulation of RA biosynthesis can be the foundation for metabolic engineering of other dietary phenolic phytochemicals from cross-pollinating, heterogeneous species.³

Proline-linked phenolic synthesis in seed sprouts

Preliminary results^{3,6,109,110,122} have provided empirical evidence for a link between proline biosynthesis and oxidation, as well as stimulation of G6PDH. In light-mediated sprout studies in pea (*Pisum sativum*), acetylsalicylic acid in combination with fish protein hydrolysates (a potential source of proline precursors) stimulated phenolic content and guaiacol peroxidase (GPX) activity during early germination with corresponding higher levels of proline and G6PDH activity.¹²³ In parallel light-mediated studies in pea, low pH and salicylic acid treatments stimulated increased phenolic content and tissue rigidity. Similarly, there was concomitant stimulation of G6PDH and proline.¹²² This work supported the hypothesis that pentose-phosphate pathway stimulation may be linked to proline biosynthesis and that modulation of a proton-linked redox cycle may also be operating through proline-linked pentose-phosphate pathway.¹²² In dark-germinated studies in pea, high cytokinin-containing anise root extracts stimulated phenolic content and antioxidant activity, which correlated with increased proline content, but inversely with G6PDH activity.¹²⁴ In further dark-germination studies in mung bean (*Vigna radiata*), dietary-grade microbial polysaccharide treatments stimulated phenolic content and enzyme activity, G6PDH and GPX compared to controls,¹²⁵ with concomitant stimulation of proline content. In addition, specific elicitors xanthan gum, yeast extract and yeast glucan stimulated antioxidant activity. In additional studies, oregano phenolic extracts were used as elicitors to stimulate phenolic content during dark germination of mung bean. Again, increased phenolic content corresponded to an increase in activity of G6PDH and GPX and phenolic-related antioxidant activity were also stimulated.¹²⁶ In studies with dark-germinated fava bean, support for the hypothesis that stimulation of proline-linked pentose-phosphate pathway would stimulate phenolic metabolism under elicitor and stress response was probed. In polysaccharide elicitor studies, gellan gum stimulated fava bean total phenolic content by 9-fold in late stages of germination with a corresponding increase in proline content and GPX activity, although the effect on antioxidant and G6PDH activity was inconclusive.³⁴ In the same fava bean system, UV-mediated stimulation of phenolic content in dark-germinated fava bean sprouts indicated a positive correlation to G6PDH and GPX activities with a concomitant increase in proline content.¹²⁷ It was further confirmed that proline analogue, A2C also stimulated phenolic content in fava bean with positive correlation to G6PDH and GPX activities as well as proline content.¹²⁸ Similar to studies in clonal shoot cultures of thyme¹⁰⁹ and oregano,¹¹⁰ the proline analogue-mediated studies in fava bean confirmed that proline over-expression was not only possible, but involved stimulating G6PDH and therefore

synthesized in plants. Investigations to date have supported the hypothesis that during development and stress response when phenolic biosynthesis is stimulated, an alternative mode of oxidative phosphorylation linked to proline metabolism may be more efficient and suitable (Fig. 2). In this alternative model, the synthesis of proline is coupled to activity of the pentose-phosphate pathway, whereby diversion of carbon flux from TCA cycle at the level of α -keto glutarate to glutamic acid drives the flux towards proline biosynthesis, which requires NADPH_2 and is supplied by the activity of the pentose-phosphate pathway. Any proton-linked redox cycling that may occur as acidification of the cytosol from the introduction of exogenous phytochemicals, microbial elicitors or true acids could also be coupled to proline-linked pentose-phosphate pathway activity and therefore stimulate activity to support co-substrate needs (NADPH_2 and sugar phosphates) of anabolic pathways, including antioxidant and defence pathways (Fig 2). In this scheme, proline serves as strong RE (alternative to NADH from Krebs/TCA cycle) for mitochondrial oxidative phosphorylation. An active ATP-producing metabolic role of proline (beyond its normally suggested role as an osmolyte) has been previously suggested.^{3,65,103} Though evidence to support the proline-linked pentose-phosphate pathway hypothesis is still emerging, the general concept from the model has already been exploited for developing phytochemicals for functional (health) food applications, especially in generating dietary phenolic antioxidants and antimicrobials. In the Mint (*Lamiaceae*) family, which is a source of many valuable phenolic phytochemicals,³ proline analogue and bacterial perturbation have been used to isolate high phenolic and rosmarinic clonal lines¹¹⁶⁻¹²¹ that can subsequently be grown as single seed origin clonal phenotypes on a large scale using vegetative propagation. In the legume family, phytochemical elicitors, acid treatment, proline precursors, proline analogues and microbial elicitors have been utilized to stimulate phenolic antioxidant-type phytochemicals during the sprouting stages.^{34,35,122-131} In the area of environmental applications microbial interaction, proline precursors and phytochemical elicitation have been utilized to enhance vigour response of germinating seeds^{122,123,130-133} and reduce problems associated with vitrification-linked adaptation of plant tissue cultures.¹³⁴⁻¹³⁷ In the area of phytoremediation, the clonal screening concept in *Lamiaceae* has been used to isolate high phenolic clonal lines that are tolerant to aromatic pollutant analogues¹³⁷⁻¹⁴² and to develop a model for pollutant (poly aromatic hydrocarbons) tolerance based on correlation between phenolic and proline content and GPX activity.¹⁴²

Model for mechanism of action of phenolic antioxidants and role of proline-linked pentose-phosphate pathway

Research efforts on phenolic phytochemical biosynthesis have focused mainly on antioxidant-type metabolites in several food plants due to relevance of major oxidation-linked chronic human diseases such as CVD, arthritis, cognition diseases (Alzheimer and Parkinson's), cancer and diabetes.¹ Phenolic antioxidants from food plants

have also been targeted as a source of antimicrobial phytochemical profiles against bacterial pathogens as an alternative and complement to antibiotics due to the increasing emergence of antibiotic resistance in such pathogens. Design of functional and health foods will take into consideration the above major health targets (oxidation-linked chronic diseases and bacterial infections). Therefore, understanding the mechanism of action of phenolic antioxidants is very important and critical for health and wellness applications.

A model has been developed for mechanism of action of phenolic metabolites based on correlation between stress-stimulated phenolic biosynthesis and stimulation of antioxidant enzyme response pathways in plant systems. In this model (Fig. 2), acid, exogenous phenolic, proline analogues and precursor combinations and microbial elicitors were used to stimulate phenolic biosynthesis and key antioxidant enzyme responses. During the phenolic response, proline content and its association to the first committed step of the pentose-phosphate pathway (G6PDH activity) was followed. Positive proline and G6PDH correlations were associated with increased phenolic content, potential polymerization of phenolics represented by GPX activity, antioxidant free radical scavenging antioxidant activity of phenolic extracts and superoxide dismutase activity.^{122,125,126,129,143} In this model (Fig. 2), elicitors through various independent and/or common pathways directly or indirectly mediate a proton-linked redox cycle in the cytosol, which is coupled to the stimulation of proline-linked pentose-phosphate pathway activity. This stimulation results in proton recycling through NADPH_2 -requiring proline biosynthesis which is replenished by the forward reactions of the pentose-phosphate pathway which generate more NADPH_2 for proline biosynthesis and other biosynthetic pathways, including phenylpropanoid and antioxidant response pathways involving SOD and peroxidases. Again, proline that is produced is then used as an alternative RE in place of NADH for driving mitochondrial oxidative phosphorylation for ATP synthesis similar to the previously described model.

Other plant and plant-fungal fermentation systems have been developed to understand the role of phenolic antioxidants and how the regulation of their synthesis and function may critically involve the proline-linked pentose-phosphate pathway. We have developed an *Agrobacterium rhizogenes*-induced root culture system wherein the natural auxin and cytokinin genes were transferred to anise (*Pimpinella anisum*) shoot explants and then naturally transformed and morphologically distinct roots were isolated.^{111,144} The proposed advantage of this system is based on the rationale that additional flux towards auxin and cytokinin pathways would over-express the proline-linked pentose-phosphate pathway (Fig. 1). In root cultures, total phenolics and a novel phenolic metabolite, epoxypseudo-isoeugenol-2-methylbutyrate (EPB), were highest in transformed root cultures and correlated with increased proline content.¹⁴⁴ Antioxidant activity was higher in late stages of root culture, but there were no significant differences between untransformed and transformed root cultures.¹⁴⁴

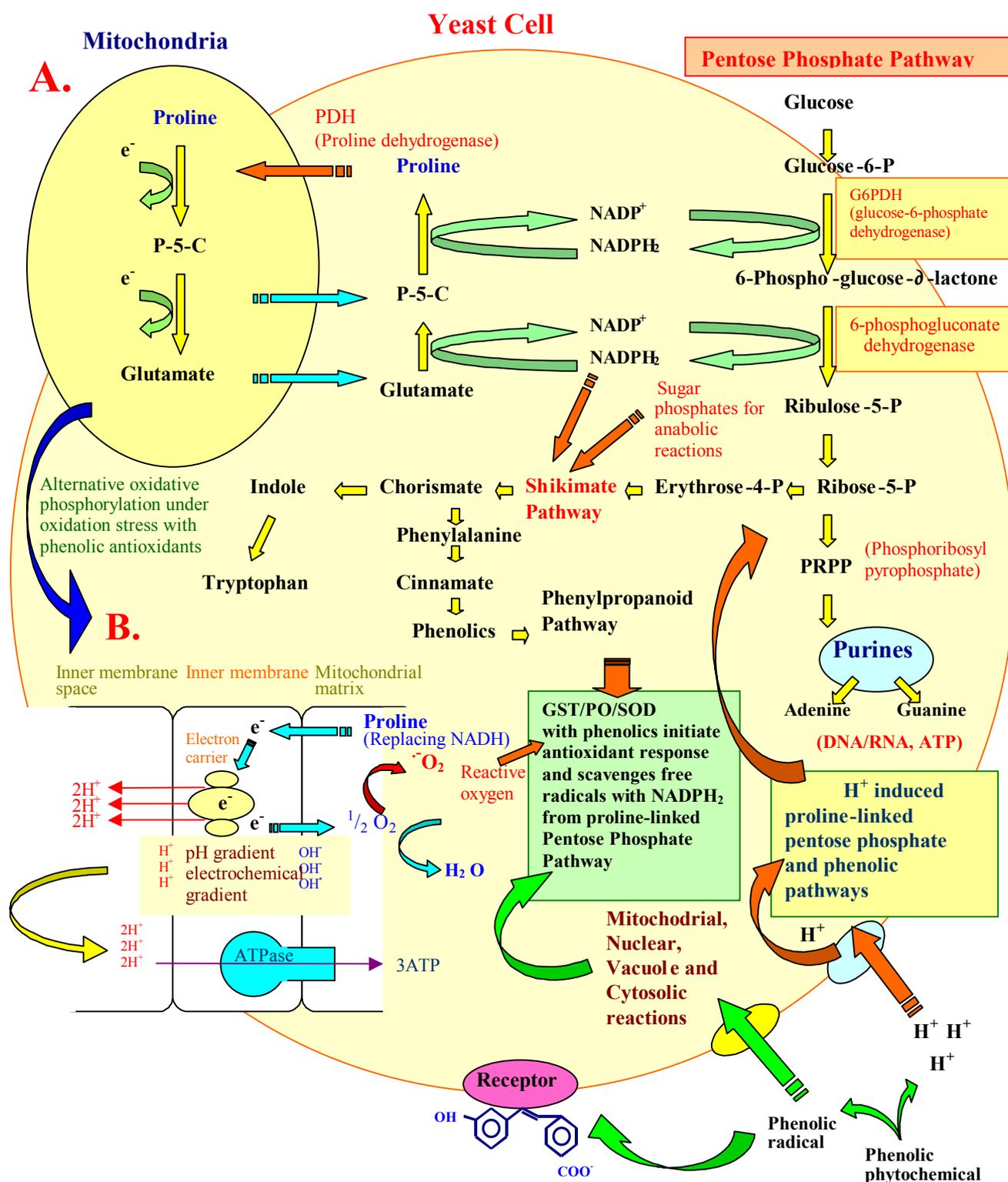


Figure 3. Extension of plant proline-linked pentose phosphate pathway model for the effect of external phenolic phytochemicals in yeast and fungal systems. (Abbreviations: P5C; pyrroline-5-carboxylate, GST; Glutathione-s-transferase, PO; peroxidase, SOD; superoxide dismutase)

Additional studies showed that elicitor treatment in the form of acetylsalicylic acid and proline precursors from likely stimulated lignification as indicated by an increase in GPX activity in transformed root cultures.¹⁴⁵ EPB synthesis in response to elicitors in transformed root cultures increased 3-6-fold. However, in the stimulated state, antioxidant activity and G6PDH activity did not vary between treated and control cultures.¹⁴⁵ Proline and proline analogues were used to stimulate somatic embryogenesis in *A. rhizogenes*-transformed root cultures.

Highest embryos were produced in response to proline and proline analogue treatments and this correlated to highest total phenolic content.¹¹¹ The proline precursors ornithine and arginine in combination with proline analogue also significantly stimulated somatic embryogenesis.¹¹¹

In another study, phenolic synthesis and antioxidant activity was investigated in snow alga, *Chlamydomonas nivalis* in response to UV light. Exposure to UV light resulted in a 5-12% increase in total phenolic content.¹⁴⁶

Free proline was not affected by UV-A, but increased markedly after UV-C exposure. Antioxidant protection increased in response to UV-A, but remained constant after UV-C exposure.¹⁴⁶ Initial summary of this research is that UV light exposure, especially in the UV-C range, can stimulate phenolic antioxidant production in aplanospores of *C. nivalis* by affecting biochemical pathways related to proline metabolism.¹⁴⁶

Solid-state bioprocessing of plant substrates using dietary fungal systems has been developed for the production of functional phenolic ingredients and enzymes for food-processing.¹⁴⁷⁻¹⁵⁰ Additionally, we have developed solid-state bioprocessing for environmental applications.¹⁴⁷⁻¹⁵⁰ In the context of solid-state production of phenolic antioxidants, we have found that dietary fungi *Rhizopus oligosporus* and *Lentinus edodes* can mobilize the phenolic metabolite ellagic acid from cranberry pomace.^{40,41} Further, we have found that water extracts containing high phenolic content have antimicrobial effects against the food-borne pathogens, *Listeria monocytogenes*, *Vibrio parahaemolyticus* and *Escherichia coli* (unpublished results). The phenolics were also antimicrobial against *Helicobacter pylori*, a pathogen linked to gastric diseases. We have now extended this fungal-mediated phenolic mobilization concept to soy-bean phenolics.¹⁵¹ We have proposed a model that under the conditions of high phenolic antioxidant mobilization, fungi, including dietary yeasts, may activate a proline-linked pentose-phosphate pathway for energy and reducing equivalent needs (Fig. 3). This would also support antioxidant protection of the fungi using the mobilized phenolics under higher oxidative stress. A proline-linked, phenolic antioxidant-protective response may also be enhanced by addition of proline and proline precursors to the growth medium. This model, if confirmed, has exciting potential applications for improving the bioprocessing abilities and stability of all fungal species used for diverse food, industrial and environmental applications.

Additional series of investigations using plant tissue cultures have been undertaken to determine whether proline precursors from hydrolyzed fish proteins can be used to stimulate plant tissue differentiation and phenolic antioxidant response. Initial results have practical applications for adaptation of plant tissues cultures and seeds in outdoor environments. Based on the observations that proline and proline analogues stimulated cytokinin-induced shoot organogenesis,¹⁰¹ fish protein hydrolysates and proline analogues were used to similarly stimulate shoot organogenesis.¹⁰² In both studies, proline content was stimulated when shoot organogenesis was stimulated. Studies in anise root cultures indicated that auxin-induced somatic embryogenesis was stimulated by proline and proline precursors (ornithine and arginine) in combination with proline analogues.¹¹¹ Based on this study, fish protein hydrolysates with high proline and glutamic acid in combination with proline analogue were used to stimulate auxin-induced somatic embryogenesis.¹⁵² In other studies, fish protein hydrolysates were used to reduce vitrification or hyperhydricity-related malformation of tissue cultures that normally reduce outdoor adaptations.^{153,154} In these studies, reduction of tissue

malformation was suggested to relate to the stimulation of phenolic response and possibly improved lignification as indicated by enhanced GPX activity.¹⁵⁴ The practical success of using fish protein hydrolysates was then extended to the improvement of seedling vigour in germinating seeds.^{123,130} The extent of improvement of seedling vigour and early seed performance was stimulated in response to fish protein hydrolysates. The improved response was correlated to phenolic response and stimulation of proline content, G6PDH and GPX, therefore suggesting a role for proline-linked pentose-phosphate and phenolic antioxidant response pathways.

Role of antioxidant response pathway in plants

Plants synthesize diverse phenolic metabolites, which are then compartmentalized and sequestered into specific organs and tissues and within organelles of specific cells within tissues depending on the nature and functional use of the compound. Many phenolic metabolites serve as antioxidants under various biological and physical stresses by minimizing oxidation-induced damage within tissues where they are produced.^{125,126,129} Plant phenolics have the potential to behave as antioxidants by trapping free radicals from various oxidative processes and, in particular, mitochondrial-linked oxidative phosphorylation. The phenolic antioxidants can function either to trap free radicals in direct interactions mediated by direct enzymatic/non-enzymatic steps, or quench free radicals through a series of coupled antioxidant enzyme defence systems^{155,156} (Fig. 4). The coupled enzymatic defence systems could involve low-molecular weight antioxidants such as ascorbate, glutathione, α -tocopherol, carotenoids and phenylpropanoids, in conjunction with several enzymes such as SOD, catalase, peroxidases, glutathione reductase and ascorbate peroxidase.^{155,157-159} In a schematic representation (Fig. 4) based on UV and ozone-stress induced chemical changes,¹⁵⁵ SOD converts the superoxide radical into H_2O_2 and O_2 . The antioxidants like ascorbate and glutathione participate in both enzymatic and non-enzymatic H_2O_2 degradation.^{155,160} Catalase converts H_2O_2 into water and O_2 , whereas peroxidase degrades H_2O_2 by oxidation of co-substrates (i.e. phenolic antioxidants).^{155,161} Other peroxidases, such as those specific to ascorbate but not to glutathione, are also observed in plants.^{155,158,162} Ascorbate peroxidase catalyzes the first step of H_2O_2 scavenging pathway by oxidizing reduced ascorbate. Monodehydroascorbate reductase and dehydroascorbate reductase catalyzes the conversion of monodehydroascorbate or dehydroascorbate to reduced ascorbate by oxidizing glutathione.¹⁵⁵ Glutathione (GSH) is regenerated by glutathione reductase in a $NADPH_2$ dependent reaction. Additionally peroxidases metabolize H_2O_2 by using phenolic metabolites as co-substrates through ascorbate-dependent pathway.^{155,163} Phenolic compounds such as flavonoids, in addition to their inherent antioxidant properties are believed to protect plants from UV stress.¹⁶⁴ These protective mechanisms are suggested to work through antioxidant response pathways involving peroxidases (as mentioned above) as well as biosynthesis of polymeric phenolics that lead to protective lignification or smaller polymers that act as antioxidants.^{127,163}

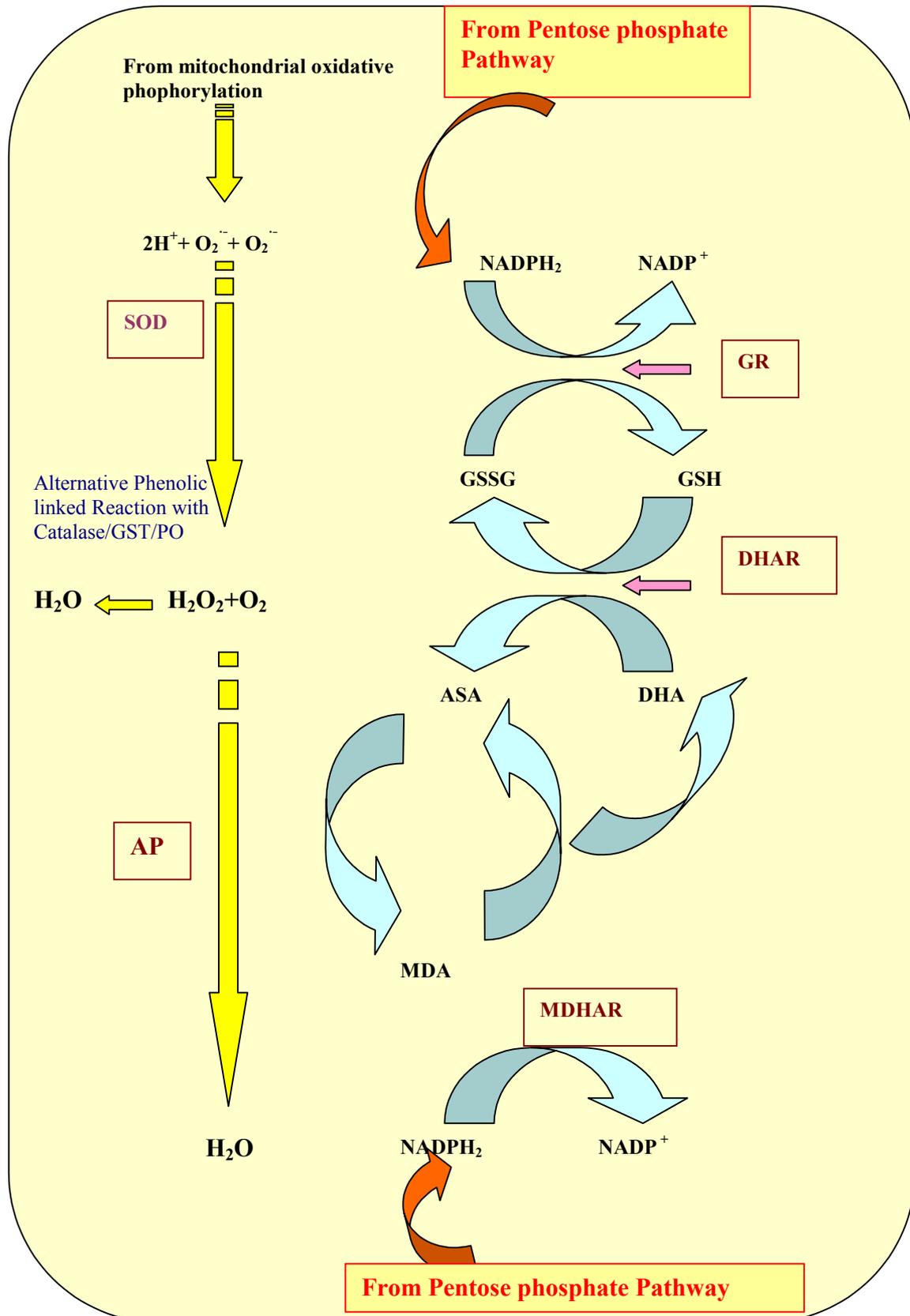


Figure 4. Model for specific steps in antioxidant response pathway in plants. (Abbreviations: SOD; superoxide dismutase, AP; ascorbate peroxidase, GR; glutathione reductase, GSSG; oxidized glutathione, GSH; reduced glutathione, DHAR; dehydroascorbate reductase, ASA; reduced ascorbate, DHA; dehydroascorbate, MDA; monodehydroascorbate, MDHAR; monodehydroascorbate reductase).

It is clear from current investigations in plant systems that the antioxidant response pathway is dependent on important NADPH₂-requiring enzymes, similar to proline biosynthesis. From our initial investigations in food plant systems,^{34,122,125-129} we have proposed that proline-linked

pentose-phosphate pathway activity could provide the NADPH₂ and sugar phosphate needs of phenolic synthesis and antioxidant pathway activity. This proposal is supported by enhanced activity of G6PDH in response to various elicitor stresses and concomitant stimulation of

phenolics, proline and GPX in several food sprout systems. In this model, proline could replace NADH from Krebs/TCA cycle as the RE for oxidative phosphorylation for ATP synthesis. The proline-coupled synthesis of phenolics and antioxidant response pathways could also serve to scavenge and protect from any free radicals that may be generated during proline-linked oxidative phosphorylation or other free radical-generating processes active in the cell during a stress-induced state. It is also conceivable, based on pH and salicylic-induced stimulation of proline-linked phenolics, G6PDH and GPX activity,¹²² that cytosolic acidification could be involved in the activation and coupling of NADPH₂-generating steps that are part of the pentose-phosphate pathway.

Role of proline in animal systems

The role of proline in human and mammalian physiology is being investigated by Phang and co-workers at the National Institutes of Health.¹⁰³ Early studies showed that activities of enzymes degrading and synthesizing proline were related to development in rat liver and kidney.¹⁶⁵ It was reported that proline oxidase/P5C dehydrogenase ratios were 25 to 50-fold higher in adult central tissue than fetal tissue indicating that proline degradation was favored in adult stages and during synthesis in fetal stages.¹⁶⁵ Another important study showed the transfer of P5C as oxidizing potential from hepatocytes to erythrocytes by inter-conversion from proline forming a redox cycle.¹⁶⁶ The resulting couple to the pentose-phosphate pathway in erythrocytes was suggested to enhance production of 5-phosphoribosyl pyrophosphate (PRPP), a key substrate for purine synthesis.¹⁶⁶ Later studies showed that P5C could stimulate glucose oxidation through pentose-phosphate pathway in cultured human fibroblasts, Chinese hamster ovary cells and rabbit kidney cells.¹⁶⁶ Further, it was shown that reducing equivalents could be transferred into mitochondria by inter-conversions of proline to P5C.^{103,104,108} From these studies, it was proposed that the shuttling of proline via P5C could function to increase ribose-5-phosphate by the oxidative limb of the pentose-phosphate pathway for PRPP and purine synthesis during growth.¹⁰⁸ In another interesting study, P5C markedly increased the activation of purine anti-metabolites, 6-thiohypoxanthine, 6-thioguanine and azathiopurine, to their respective nucleotides in intact human erythrocytes,¹⁶⁷ and later confirmed that P5C can stimulate PRPP to support purine biosynthesis in erythrocytes.¹⁶⁸ From these studies it is clear that as a redox couple, proline and P5C provide a mechanism for the inter-compartmental and intercellular transfer of redox potential.¹⁰³ The transfer of the redox potential alters the ratio of NADP⁺/NADPH₂, which can then meet the needs of anabolic pathways by producing more NADPH₂. It was also confirmed that even though reduction of P5C is important for the transfer of redox potential, the metabolic inter-conversions of proline, ornithine and glutamate might also play an important role.¹⁰³ The end point of the animal studies showed that this redox cycle favoured the formation of purine ribonucleotides.¹⁰³ From these mammalian system studies, it was hypothesized that synthesis of plant phenolic metabolites may be linked to the proline-linked pentose phosphate pathway³ (Fig. 1). In the

animal model, proline is synthesized by a series of reduction reactions from glutamate. In this sequence, P5C and proline function as a redox couple and are known as metabolic regulators.^{103,104,108} During respiration, oxidation reactions produce hydride ions, which aid reduction of P5C to proline in the cytosol. Through activity of proline dehydrogenase /proline oxidase,^{103,105} proline could enter mitochondria and support oxidative phosphorylation. The reduction of P5C to proline in the cytosol increases NADP⁺, which activates G6PDH, an enzyme that catalyzes the rate-limiting step of the pentose-phosphate pathway. Proline synthesis is therefore hypothesized and partly shown to regulate pentose-phosphate and purine pathways in erythrocytes¹⁰⁶ and cultured fibroblasts.¹⁰⁷ Proline synthesis was shown to stimulate purine metabolism via ribose-5-phosphate, which affects cellular physiology and therefore function.^{103,108} A role of proline metabolism in modulation of cellular physiology has been further confirmed in p53-dependent initiation of apoptosis in a colorectal cancer cell line.¹⁶⁹ In this study, p53-dependent initiation of apoptosis was accompanied by the induction of proline oxidase, a mitochondrial enzyme catalyzing the conversion of proline to P5C with concomitant transfer of electrons to cytochrome c.¹⁶⁹ Based on other studies, the up-regulation of proline oxidase during p53-dependent apoptosis has been suggested, indicating a role in redox regulation.¹⁶⁹⁻¹⁷¹ In another interesting study, proline oxidase was up-regulated in a p53-sensitive bladder carcinoma cell line but not in a p53-resistant cell line.¹⁷² Further, it was shown that P5C generated by proline oxidase, inhibited the proliferation and survival of these bladder carcinoma cells and induced apoptosis in both cell lines.¹⁷² This study directly implicated proline oxidase and proline/P5C interconversions in p53-induced growth suppression and apoptosis.¹⁷² A recent study has further indicated that proline oxidase induces apoptosis in tumour cells and that its expression is frequently absent or reduced in renal carcinomas.¹⁷³ In the context of other diseases, molecular genetics studies based on patients with adult or childhood onset of schizophrenia strongly suggest that genetic variations in proline oxidase gene may increase the risk of susceptibility to schizophrenia.¹⁷⁴

Role of phenolic antioxidant metabolites and proline-linked pentose-phosphate pathway in human health: a hypothesis

Preliminary studies in food-grade clonal herb systems³ and legume sprouts^{125,126,129} led to the development of the model that activity of proline-linked pentose-phosphate pathway is important for stress-induced phenolic biosynthesis and that this stimulation of phenolics may be closely linked to stimulation of antioxidant response pathways.^{125,126,129} Further research has indicated that the proline biosynthesis pathway coupled to stress-induced antioxidant response pathways could be also stimulated in legume sprouts using exogenous treatment of phenolic extracts from clonal oregano.^{35,126,130} Phenolic extracts from these clonal oregano lines have high free-radical scavenging activity. Proline-linked stimulation of antioxidant response pathways may also be stimulated by low

pH and salicylic acid.¹²² Further, exogenous seed treatment with oregano phenolic antioxidant extracts enhanced endogenous phenolic content, GPX activity, and consequently, enhanced seedling vigour during germination.¹³⁰ From these initial plant studies and plant model (Fig. 2), a human/mammalian cell model has been developed (Fig. 5) wherein a proton donation by phenolic antioxidants at the outer plasma membrane initiates a proton/hydride ion influx into the cytosol which activates an antioxidant response through the stimulation of the proline-linked pentose-phosphate pathway. Demand for NADPH₂ by stimulated proline biosynthesis also drives the production of precursors for phenolic (only in plants/fungi), purine and antioxidant pathways. In this model, proline can be used as a RE to support oxidative phosphorylation for ATP synthesis. Using this model, we have developed several phenolic over-expressing plant systems for functional food and agro-environmental applications. The optimized phenolic phytochemical profiles can be used as antioxidants and antimicrobials in biological systems and have implications for human health and wellness.

From the assumptions based on the animal antioxidant response model (Fig. 6) and the plant antioxidant response model (Fig. 2 & 4), a new, integrated model for the mechanism of action of phenolic antioxidants for human health involving the proline-linked pentose-phosphate pathway has been proposed (Fig. 5). It is clear that many of the major diseases afflicting humanity today in an age of increasingly sufficient and, perhaps, excess calories, are oxidation-linked chronic diseases such as cancer, CVD, arthritis, cognition diseases, and diabetes. Oxidation-linked immune dysfunction and the inability to fight pathogenic infection under a very low calorie and protein diet remains a problem in several parts of the developing world and needs continued and serious attention. Oxidation-linked and infectious diseases involve free-radical reactions. Free radicals are potential carcinogens because they can facilitate mutagenesis, tumour promotion and progression.¹⁷⁵⁻¹⁷⁷ For example, in the case of cardiovascular diseases, free radicals are implicated in the pathogenesis of atherosclerosis, which is characterized by the hardening of the arterial wall.^{175,178,179} Rheumatoid arthritis, a systemic autoimmune disease characterized by chronic joint inflammation with infiltration of macrophages and activated T cells. Production of free radicals at sites of inflammation is thought to contribute to the pathogenesis.^{175, 180,181} Free radicals are implicated in the pathogenesis of Alzheimer disease.^{175,182} As significant amounts of lipid peroxidation in brain tissues have been observed and may help explain the progressive decline in cognition function and excessive neuronal loss in afflicted patients. The brain tissue shows numerous amyloid plaques. Free radicals have been implicated in Diabetes Mellitus.^{175,183,184} In this disease, oxidative stress is associated with a pro-oxidative shift of glutathione redox state in the blood.^{175,185} Elevated glucose levels are associated with increased production of free radicals by several different mechanisms.^{175,186,187}

All of the above diseases are in part diet- and lifestyle-influenced and improvement of diet is important in the prevention and management of these diseases. It is evident that a variety of plant-based phenolic antioxidants

have a positive impact on the prevention and modulation of various oxidation-linked diseases^{10,18,19,43,188,189} and must be considered an important part of the dietary management of these diseases. Currently the modes of action and early stage effects of these phenolic antioxidants in positively modulating and preventing various diseases are not completely clear. Extensive research is underway to ascertain how free radicals modulate physiological control of cell function at the level of cell proliferation and deterioration¹⁷⁵ and at the level of gene expression.¹⁹⁰ Phenolic antioxidants have been targeted to control the free radical-linked cellular deterioration that can lead to major oxidation-linked chronic diseases.

One way to develop diet-based interventions is through design of functional foods (conventional foods with clinically defined health promoting components) based on the understanding of phenolic antioxidant biosynthesis in food plants (Fig. 2) and the effect of such phenolic antioxidants in human and mammalian systems (Fig. 5). In order for diet-based interventions (through functional foods) to be effective, it is also important to understand the early stage modes of action of these functional compounds and how to deliver them at consistent levels and with no toxicity problems. In the antioxidant response model for human health, proposed herein a consistent and defined phytochemical profile of phenolic antioxidants can be developed using clonal shoot, sprout and fermented systems using various dietary botanicals as discussed earlier (Fig. 2). In the human model (Fig. 5), the early stage mode of action of these phenolic antioxidants in human cells has parallels to the models for plant and fungal systems (Fig. 2, 3 & 5), but within the scope of human cellular physiology, function, and diversity. By this model, plant phenolic antioxidants similarly initiate an inward proton flux at the outer human cell membrane, which increases the cytosolic proton/hydride ion concentration and activates the proline-linked pentose-phosphate pathway. Some phenolic antioxidant radicals, depending on their size, may penetrate the plasma membrane along with the proton/hydride ion flux (co-transport) into the cytosol. The cytosolic proton/hydride ion flux then drives proline-linked pentose-phosphate pathway generating NADPH₂, sugar phosphates for anabolic reactions and proline as an alternative RE to generate ATP via oxidative pentose-phosphate pathway. The products of the pentose-phosphate pathway are important for purine biosynthesis and for stimulating antioxidant enzyme response pathways in conjunction with action of the dietary phenolic antioxidants (Fig. 6). The control of free-radicals that is likely associated with proline or TCA cycle generated NADH-linked mitochondrial oxidative phosphorylation at this early stage could have a positive effect on any subsequent oxidation-linked cellular deterioration and consequent oxidation-linked chronic disease manifestations. Other roles for phenolic radicals that penetrate the membrane could involve: a) stability and protection of organelle membranes and proteins from free-radical damage; b) participation in the antioxidant response pathway to quench superoxide and peroxide radicals; c) protection of DNA and protein stability and/or d) stimulation of proline-linked pentose-phosphate pathway activity to

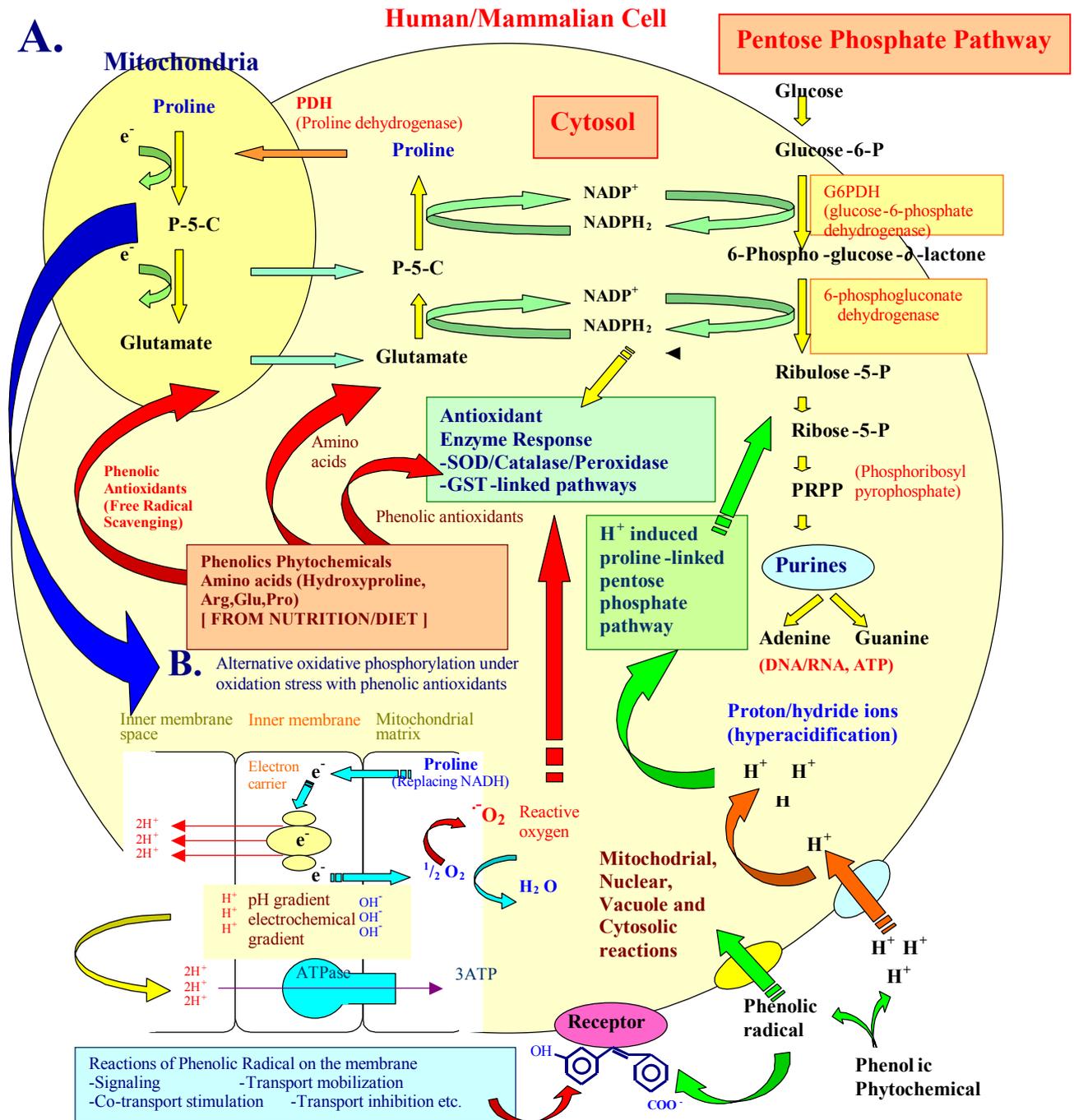


Figure 5. Extension of plant proline-linked pentose phosphate pathway model for the effect of external phenolic phytochemicals in human and mammalian systems. (Abbreviations: P5C; pyrroline-5-carboxylate, GST; Glutathione-s-transferase, SOD; superoxide dismutase)

satisfy demand for NADPH₂ in reactions involving penetrating phenolic radicals.

In specific cases where phenolic radicals cannot normally penetrate the outer plasma membrane, other conceivable roles could include: a) stability and protection of the outer membranes and membrane proteins from free-radical damage, b) modulation of membrane transport, c) inhibition of specific membrane proteins, including those involved in PMF and electron transport chain in Prokaryotes, d) modulation of signal transduction, e) modulation of membrane receptors, f) co-transport with H⁺, sugars and/or amino acids and g) passive membrane transport through damaged membranes. An inward proton flux to the cytosol could be created even without phenolic radical penetration, which

then could stimulate the proline-linked pentose-phosphate pathway and couple its action to the various reactions and roles that may be initiated and modulated through interactions of phenolic radicals at the outer plasma membrane.

Implications of phenolic antioxidants as antimicrobials

Current theory and emerging data suggests that eukaryotes evolved from prokaryotes.¹⁹¹ Genetic evidence suggests that plant organelles like chloroplasts, mitochondria, and even vacuoles may have origins as free prokaryotes. The compartmentalized organization and cellular differentiation (into tissues) of plants apparently evolved in terrestrial environments about 500 million years ago. It is likely that through the millennia, plastic

Pathways of reactive oxygen species (ROS) and phenolic-linked antioxidant response

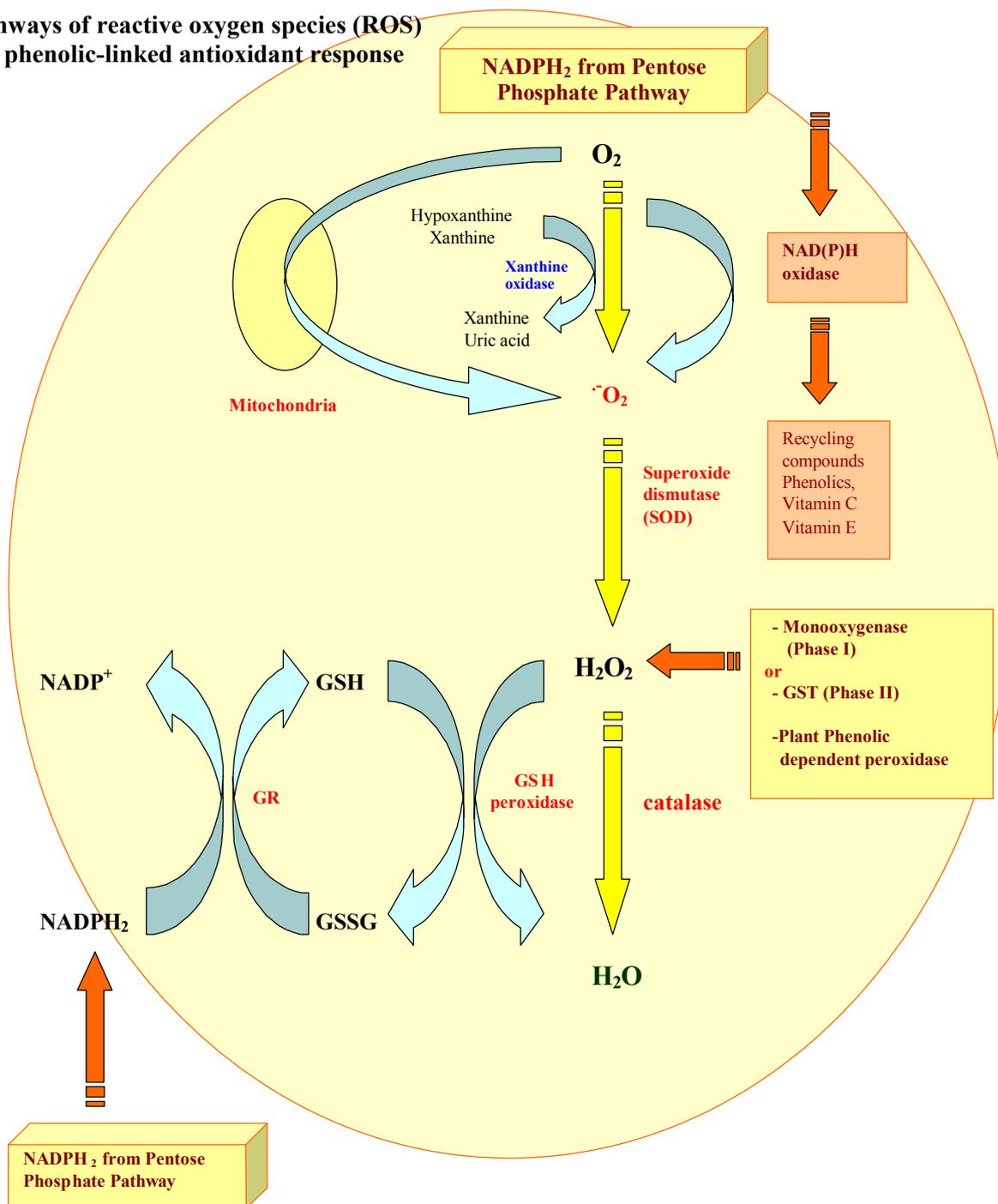


Figure 6. Model for specific steps in antioxidant response pathways in human and mammalian systems. (Abbreviations: SOD; superoxide dismutase, GST; glutathione-s-transferase, GR; glutathione reductase, GSH peroxidase; glutathione peroxidase, GSSG; oxidized glutathione, GSH; reduced glutathione)

plant species constantly interacted with many microorganisms and environmental stresses, especially UV radiation and oxidative stress. The speculation is that constant environmental stress in many ways may have shaped the antimicrobial and phenolic antioxidant responses of plants through the evolutionary process. Natural selection of plants under various changing environmental conditions over millions of years, in many critical ways (directly or indirectly) may have shaped the current mammalian systems, including human nutrition, health, and dietary evolution.

It is from these assumptions that we are exploring the mechanism of antimicrobial action of plant phenolics on prokaryotic pathogens and the activation and maintenance of plant and eukaryotic antioxidant responses, at a similar phenolic concentration needed for bacterial inhibition. Plant phenolics and related synthetic food-grade phenolics have been suggested to inhibit bacterial pathogens (particularly at low pH) by disrupting the proton motive force (PMF). This implies that regulation of H^+ -ATPases and co-factors at the external membrane is critical, and may be a logical point to study the mechanism of inhibition by plant phenolics. Additionally, plant phenolics

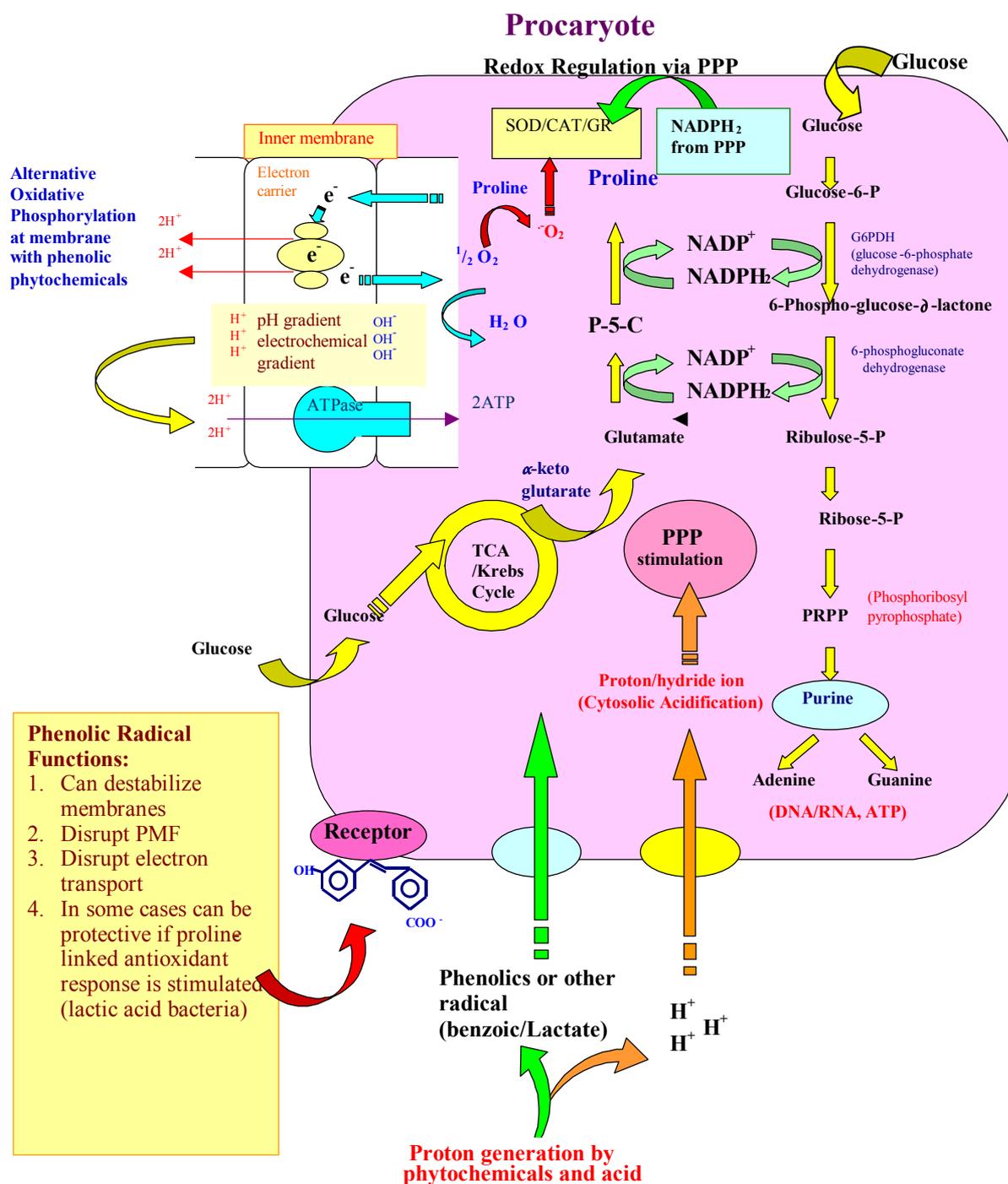


Figure 7. Extension of plant proline-linked pentose phosphate pathway model for the effect of external phenolic phytochemicals in prokaryotic bacterial systems. (Abbreviations: P5C; pyrroline-5-carboxylate, SOD; superoxide dismutase, CAT; catalase, GR; glutathione reductase, PPP; pentose phosphate pathway; Krebs {TCA-tricarboxylic acid} cycle).

may disrupt the electron transport chain or destabilize the plasma membrane.¹⁹² At the same time it is important to investigate the role of plant H^+ -ATPases at both the external membrane and organelle (chloroplast, mitochondria and vacuolar tonoplast) membrane levels to determine their potential involvement in modulation of a phenolic radical and proton-linked redox cycle. This cytosolic phenolic radical/proton-linked redox cycle (as described earlier) is hypothesized to activate the proline-linked pentose-phosphate pathway, to utilize proline as an alternative RE for mitochondrial ATP synthesis, and to generate precursors and co-substrates (NADPH₂ and sugar phosphates) for anabolic reactions, including a phenolic-

linked antioxidant response and purine synthesis.^{3,122} The genetic comparisons of H^+ -ATPase alleles from bacterial and eukaryotes could provide clues towards understanding their susceptibility to plant phenolics in bacteria and the antioxidant response-linked tolerance of plants and other eukaryotes. Such knowledge could facilitate the development of better structure-function strategies for the development of better botanical phenolic profiles (i.e herb and legume clonal extracts) that may provide multiple beneficial activities. A better conceptual understanding of phenolic antimicrobial effects in bacteria and antioxidant responses in eukaryotic hosts could also facilitate the development of diet-based nutritional and

functional food strategies to control bacterial pathogens with reduced potential for antibiotic resistance due to the concerted effect of a phenolic profile, instead of a single compound, as in case of antibiotics. Such an integrated approach involving phenolic phytochemicals has excellent potential to compliment current antimicrobial strategies.

Taking into account the above rationale, a phenolic antimicrobial activity model (Fig. 7) that incorporates the role of proline-linked pentose-phosphate pathway provides a better perspective than current putative antimicrobial models for phytochemical action.¹⁹² The phytochemical profiles that have the potential to inhibit pathogenic microorganisms¹⁹²⁻¹⁹⁴ contain secondary metabolites that are defensive and inducible anti-microbials produced against both invading pathogens and stress. Therefore the methods for exploiting them must take this also into account. In certain cases, the induction is associated with action of diphenolic oxidases and resulting modified compounds can have antimicrobial activity.¹⁹⁵ In some cases, dihydroxy phenolics are oxidized to highly reactive quinones, which can interact with proteins of the invading pathogens and form melanoid polymers.¹⁹² As the compounds responsible for the enzymatic browning reaction of cut fruits and vegetables and an intermediate in melanin pigment production in humans,¹⁹² quinones are a source of stable free-radicals and complex irreversibly with nucleophilic amino acids¹⁹⁶ leading to inactivation of proteins and loss of function.¹⁹² Therefore, the potential antimicrobial benefits of quinones are substantial.¹⁹² Potential targets for inhibition in the bacterial cell are surface adhesions, cell wall polypeptides, and membrane-bound enzymes.¹⁹² In herb species of the *Lamiaceae* family, phenolic derivatives are largely responsible for antimicrobial activity.^{4,197} Thymol, present in the essential oil of thyme, oregano, savory, sage and related species, has excellent antimicrobial activity. The essential oil containing thymol can inhibit *Vibrio parahaemolyticus*.¹⁹⁸ The addition of 0.05% of alcoholic extracts of thyme can inhibit the growth of *Staphylococcus aureus*.¹⁹⁹ Sage extract was inhibitory to *Bacillus cereus* and *S.aureus*.²⁰⁰ Rosemary extract of 0.1% substantially inhibited the growth of *S.aureus* and *Salmonella typhimurium*.²⁰¹ Hydroxy-cinnamic acid derivatives such as caffeic acid, ferulic acid and *p*-coumaric acid inhibited *E.coli*, *S.aureus* and *B.cereus*.²⁰² Polymeric phenolics, such as tannins, were inhibitory toward *Listeria monocytogenes*, *E.coli*, *S.aureus*, *Aeromonas hydrophila* and *Streptococcus faecalis*.²⁰³ Hydroxylated phenols, such as catechol and pyrogallol, are known to be toxic to microorganisms.¹⁹² The site and number of hydroxyl groups is linked to the antimicrobial effect and in some cases more oxidized forms are more inhibitory.^{204,205}

From all the above studies it is evident that the mode of action of phenolics against bacterial pathogens has not been clearly defined or understood and many modes of action have been suggested. One major model has focused on the suspected changes in membrane permeability through membrane-localized hyperacidity, which may affect PMF across the membrane resulting in energy depletion.^{206,207} Another has proposed that enzyme inhi-

bition by oxidized compounds through reaction with enzyme sulfhydryl groups or through non-specific interactions with membrane proteins may explain the inhibition.^{192,208} Even with better models on the mechanism of action, another major limitation of using many phytochemicals is that they are derived from mixed heterogeneous genetic sources and consistency cannot be guaranteed.^{3,4} Now, with the emergence of antibiotic resistance from overuse of single antibiotics new strategies incorporating plant-based antimicrobials are both promising¹⁹² and essential.⁴ However, plant-based antimicrobial strategies will require a better model to understand the mechanism of antimicrobial action involving consistent profiles of phytochemicals.

An effective strategy proposed in this paper focuses on the hypothesis that high-antioxidant phenolics from single seed origin clonal lines of herbs, sprouted legumes, and fermented fruits would have excellent antimicrobial potential. Single seed high-antioxidant phenolic profiles have been screened, evaluated for antioxidant efficacy, and targeted to inhibit various food-borne pathogens and chronic human infections, such as those by peptic ulcer-causing *Helicobacter pylori*. The above strategy addresses both the concept of phytochemical consistency through the use of clonal lines and stress (elicitor)-based inducible phenolic antioxidants from various developmental phases of growth and also the putative impact on antimicrobial potential. Therefore, using our strategy for developing consistent and inducible phytochemical profiles, an alternative and more robust model (Fig. 7) for a mechanism of antimicrobial action incorporating the role of proline-linked pentose-phosphate pathway have been proposed. The mechanism of action of phenolic phytochemicals that may operate in prokaryotes involves the use hyperacidification (protons) from acids and phenolic metabolites and transport protons inside the cell (Fig. 7), by more acid-tolerant prokaryotes (lactic acid bacteria and some moderately acid tolerant Gram negative pathogens like *H. pylori*, *Escherichia coli* and *Salmonella*) either passively or through H⁺-transport membrane proteins. Such acid-tolerant prokaryotes may stimulate redox cycling through the proline-linked pentose-phosphate pathway and use proline (like the mitochondria of eukaryotes) as an alternative RE for ATP synthesis at the single outer plasma membrane with oxygen as the terminal electron acceptor. As mentioned before, the process of proline biosynthesis could also be coupled to the pentose-phosphate pathway for NADPH₂ recycling and to make sugar phosphates for all anabolic needs. By this model, acid-tolerant microorganisms could efficiently manage the ATP needs from stress-increased oxidative phosphorylation through coupling to pentose-phosphate pathway activity, while recycling excess proton flux from hyperacidification. Proline-linked oxidative phosphorylation could excrete excess protons outside the plasma membrane and augment the generation of PMF for ATP synthesis. This proline-linked metabolism model may occur in acid- and phytochemical-tolerant lactic acid bacteria (Gram-positive) and in moderately acid-and phytochemical-tolerant Gram-negative bacteria but is less likely in other acid-and phytochemical-susceptible Gram-positive bacteria.

One way to prove this model would be to determine if proline over-producing mutants are more acid-tolerant and if this tolerance is associated with increased generation of NADPH₂. An important difference in case of prokaryotes is that they have only one outer plasma membrane and no organelles for metabolic adjustment. Depending on the type of membrane modifications that occur between various bacterial species, phenolic radicals may negatively or positively effect membrane related functions, including transport, signalling, receptor modification and energy metabolism. Membrane-related modulation of metabolism could be closely linked to the cytosolic proton-linked modulation of proline-linked pentose-phosphate pathway. Based on this model, it is likely that Gram-positive bacteria (excluding lactic acid bacteria) would be most susceptible, followed by Gram-negative bacteria, and then by acid-tolerant lactic acid bacteria, likely being more tolerant and actually protected by phenolic phytochemicals (as antioxidants).

In eukaryotic microorganisms like yeasts, phytochemical-based inhibition is likely more challenging based on the above model. In yeasts, proton flux from acids and phytochemicals may be managed as proposed for mammals and plants, though stimulation of the proline-linked pentose-phosphate pathway (Fig. 3). Such action would support antioxidant response pathways. The implication is that, designing antimicrobial strategies for controlling yeast infections using phytochemicals becomes more challenging. For control of yeast and external fungal infections, it may be more feasible to develop phytochemicals, such as phenolics and terpenes, that disrupt external membrane transport, and to target the disruption of electron transport chain in the mitochondria only in cases where the phytochemical can penetrate the outer membrane. Alternatively, through modulation of proline-linked pentose phosphate pathway, phenolic antioxidants from food plants could be ideal for improving the stability of various yeasts and fungal systems used in food processing (i.e alcoholic beverages and dairy products) and industrial applications.

Summary and unified theory for role of proline-linked pentose-phosphate pathway in plant, animal and microbial systems

When considering the genetic, cellular and tissue divergence of various biological systems and adaptations due to environmental pressures and natural selection we must also recognize the commonalities of certain bio-chemical responses related to oxidation-linked cellular manifestations and function. During oxidation-linked stress and disease conditions, an alternative mode to regulate the pentose-phosphate pathway, as well as ATP synthesis by oxidative phosphorylation, through proline may help biological systems. Such regulation could help to efficiently manage energy needs while supporting the NADPH₂ and sugar phosphate requirements of biosynthetic pathways, including the antioxidant, antimicrobial, stress and adaptogenic, immune, and, consequently disease/health responses. In this unified model, all early responses under any kind of oxidation stress, including infectious diseases, are universally modulated through the proline-linked pentose-phosphate pathway. This inte-

grated pathway would support antioxidant response pathways to manage each stressed state depending on the biological system, and human health/disease states that are dictated by specific oxidation stresses coupled to nutritional and environmental factors.

In summary, a strategy has been developed to generate consistent profiles of phenolic antioxidants from food plants to design functional health foods for improved diet as a means to potentially manage (through prevention) oxidation-linked chronic and infectious diseases. The strategy to generate consistent phenolic antioxidant profiles has driven the development of plant system models, wherein synthesis of ATP, RE, NADPH₂ and sugar phosphates for cellular metabolism is regulated through an alternative proline-linked pentose-phosphate pathway. The empirical insights into this alternative pathway have already been exploited for many applications in many plant species. Examples include: 1) screening high phenolic antioxidant producing food-grade clonal herbs, 2) development of biological, biochemical and stress elicitation methods to stimulate phenolic antioxidants in sprout systems, 3) development of food-grade fungal bioprocessing to generate consistent phenolic antioxidants from botanical substrates, 4) strategies for developing consistent phytochemical profiles that can be targeted against bacterial pathogens and elucidation of possible mechanisms of action of phytochemicals through the bacterial proline-linked pentose-phosphate pathway, 5) environmental applications of plants via generation of high-phenolic clonal systems for phytoremediation of aromatic pollutants, and 6) environmental applications in plants utilizing the proline-linked pentose-phosphate pathway for enhanced environmental adaptation of transplanted seedlings from tissue culture or greenhouse systems.

Advancement of this concept by further research into the alternative proline-linked pentose-phosphate pathway has numerous implications for 1) understanding the mechanism of action of phenolic antioxidants from plants in mammalian and human systems; 2) understanding whether the mechanism of action of phenolic antioxidants in mammalian and human systems as proposed in this paper is regulated through the alternative proline-linked pentose phosphate pathway; 3) understanding whether phenolic antioxidants positively modulate and prevent oxidation-linked diseases through proline-linked pentose-phosphate pathway; 4) understanding whether phenolic antioxidants from plants can control bacterial infections and whether the mechanism of action involves the proline-linked pentose-phosphate pathway; 5) understanding whether phenolic antioxidant-based functional foods can be designed for managing major oxidation-linked diseases such as diabetes, CVD, inflammatory diseases, cognition diseases and cancer; 6) understanding whether phenolic antioxidant-based functional foods can be designed for managing chronic bacterial-infection-related diseases such as ulcer, urinary tract infection and dental caries, as well as food-borne bacterial infections, and whether this can complement or reduce microbial-based single antibiotic use; 7) understanding whether phenolic antioxidant-based functional foods can be designed using probiotics and yeasts, and whether the

proline-linked pentose-phosphate pathway is important for their stability in food carrier systems and in humans; 8) understanding whether phenolic antioxidants can be used for improving bioprocessing qualities and stability of yeast and bacterial acid-fermented foods, and in such cases, what is the health benefit to humans, animals and other biological systems; 9) understanding the interactions and proper balance between plant-based phenolic antioxidants and protein foods from various sources such as legumes, fish and various meats in the human diet and whether the mechanism of action is controlled through the proline-linked pentose-phosphate pathway; 10) understanding the interactions of plant-based phenolic antioxidants with carbohydrates (particularly starch and soluble sugars) and lipids (with saturated fatty acids) and whether the mechanism of action is controlled through the proline-linked pentose-phosphate pathway; 11) understanding whether liver detoxification pathways are coupled to antioxidant response pathways; and 12) relevance to designing plant and microbial systems for Advanced Life Support and Food systems in NASA and International Space projects.

Acknowledgements

The authors highly appreciate the contributions of Patrick McCue for critical reading of the manuscript and Yuan-Tong Lin for developing graphics for all figures.

References

1. Wahlqvist ML. Chronic disease prevention: A life cycle approach which takes account of the environmental impact and opportunities of food, nutrition and public health policies—the rationale for an eco-nutritional disease nomenclature. *Asia Pac J Clin Nutr* 2002; 11(S): S759-S762.
2. Wahlqvist ML, Wattanapenpaiboon N, Kannar D, Dalais F, Kouris-Blazos A. Phytochemical deficiency disorders: inadequate intake of protective foods. *Current Therapeutics* 1998; July: 53-60.
3. Shetty K. Biotechnology to harness the benefits of dietary phenolics; focus on Lamiaceae. *Asia Pac J Clin Nutr* 1997; 6:162-171.
4. Shetty K, Labbe RL. Food-borne pathogens, health and role dietary phytochemicals. *Asia Pac J Clin Nutr* 1998; 7: 270-276.
5. Shetty K. Phytochemicals: Biotechnology of phenolic phytochemicals for food preservatives and functional food applications. In: Francis FJ, ed. *Wiley Encyclopedia of Food Science and Technology*, 2nd Edition. New York: Wiley Publishers, 1999; 1901-1909.
6. Shetty K. Biosynthesis of rosmarinic acid and applications in medicine. *J Herbs Spices and Medicinal Plants* 2001; 8: 161-181.
7. Shetty K, McCue P. Phenolic antioxidant biosynthesis in plants for functional food application: Integration of Systems Biology and Biotechnological Approaches. *Food Biotechnology* 2003; 17:67-97.
8. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Rad Res* 1995; 22: 375-383.
9. Hertog MGL, Hollman PCH, Kattan MB. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J Agric Food Chem* 1992; 40: 2379-2383.
10. Hertog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, Pekkarinen M, Simic BS, Toshima H, Feskens EJM, Hollman PCH, Kattan MB. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch Intern Med* 1995; 155: 381-386.
11. Jorgensen LV, Madsen HL, Thomsen MK, Dragsted LO, Skibsted LH. Regulation of phenolic antioxidants from phenoxy radicals: An ESR and electrochemical study of antioxidant hierarchy. *Free Radical Res* 1999; 30: 207-220.
12. Paganga G, Miller N, Rice-Evans CA. The polyphenolic contents of fruits and vegetables and their antioxidant activities. What does a serving constitute? *Free Radical Res* 1999; 30: 153-162.
13. Foti M, Piattelli M, Amico V, Ruberto G. Antioxidant activity of phenolic meroditerpenoids from marine algae. *J Photochem Photobiol* 1994; 26: 159-164.
14. Huang MT, Lysz T, Ferraro T, Conney AH. Inhibitory effects of curcumin on tumor promotion and arachidonic acid metabolism in mouse epidermis. In: Wattenberg L, Lipkin M, Boone CW, Kelloff GJ, eds. *Cancer chemoprevention*. Boca Raton, FL: CRC Press, 1992; 375-391.
15. Osawa T, Sugiyama Y, Inayoshi M, Kawakishi S. Antioxidant activity of tetrahydrocurcuminoids. *Biosci Biotechnol Biochem* 1995; 59:1609-1612.
16. Lim GP, Chu T, Yang F, Beech W, Frauschy SA, Cole GM. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *J Neuroscience* 2001; 21: 8370-8377.
17. Jitoe A, Masuda T, Tengah GP, Suprapta DN, Gara IW, Nakatani N. Antioxidant activity of tropical ginger extracts and analysis of the contained curcuminoids. *J Agric Food Chem* 1992; 40: 1337-1340.
18. Masuda T, Jitoe A. Antioxidative and anti-inflammatory compounds from tropical gingers: Isolation, structure determination, and activities of cassumunins A, B and C, new complex curcuminoids from *Zingiber cassumunar*. *J Agric Food Chem* 1994; 42: 1850-1856.
19. Peake PW, Pussell BA, Martyn P, Timmermans V, Charlesworth JA. The inhibitory effect of rosmarinic acid on complement involves the C5 convertase. *Int J Immunopharmac* 1991; 13: 853-857.
20. Ruby AJ, Kuttan G, Babu KD, Rajasekharan KN, Kuttan R. Anti-tumour and antioxidant activity of natural curcuminoids. *Cancer Lett* 1995; 94: 79-83.
21. Singh SV, Hu X, Srivastava SK, Singh M, Xia H, Orchard JL, Zaren HA. Mechanism of inhibition of benzo[a]pyrene-induced forestomach cancer in mice by dietary curcumin. *Carcinogenesis* 1998; 19: 1357-1360.
22. Khar A, Ali AM, Pardhasaradhi BV, Begum Z, Anjum R. Antitumor activity of curcumin is mediated through the induction of apoptosis in AK-5 tumor cells. *FEBS Lett* 1999; 445: 165-168.
23. Inano H, Onoda M, Inafuku N, Kubota M, Kamada Y, Osawa T, Kobayashi H, Wakabayashi K. Potent preventive action of curcumin on radiation-induced initiation of mammary tumorigenesis in rats. *Carcinogenesis* 2000; 21: 1835-1841.
24. Hutchins AM, Slavin JL, Lampe JW. Urinary isoflavonoid phytoestrogen and lignan excretion after consumption of fermented and unfermented soy products. *J Am Dietetic Assoc* 1995; 95: 545-551.
25. Messina MJ. Legumes and soybeans: Overview of their nutritional and health effects. *Am J Clin Nutr* 1999; 70 (Suppl): 439S-450S.

26. Nagata C, Takatsuka N, Kawakami N, Shimizu H. A prospective cohort study of soy product intake and stomach cancer death. *Br J Cancer* 2002; 87: 31-36.
27. Kanazawa K, Kawasaki H, Samejima K, Ashida H, Danno G. Specific desmutagens and (antimutagens) in oregano against a dietary carcinogen, Trp-P-2 are galagin and quercetin. *J Agric Food Chem* 1995; 43: 404-409.
28. Winterhoff H, Gumbinger HG, Sourgens, H. On the antiogonadotropic activity of *Lithospermum* and *Lycopus* species and some of their phenolic constituents. *Planta Medica* 1988; 54: 101-106.
29. Harbone JB, Baxter H. Phenylpropanoids. In: Harbone JB, Baxter H, eds. *Phytochemical Dictionary: A Handbook of Bioactive Compounds from Plants*. London-Washington DC: Taylor and Farnois, 1993: 472-488.
30. Howell AB, Vorsa N, Der Marderosian A, Foo LY. Inhibition of adherence of P-fimbriated *Escherichia coli* to uroepithelial-cell surfaces by proantho-cyanidin extracts from cranberries. *N Engl J Med* 1998; 339: 1085-1086.
31. Howell AB, Foxman B. Cranberry juice and adhesion of antibiotic-resistant uropathogens. *JAMA* 2002; 287:3082-3083.
32. Guggenheim S, Shapiro S. The action of thymol on oral bacteria. *Oral Microbiol Immunol* 1995; 10: 241-246.
33. Himejima M, Kubo I. Fungicidal activity of polygodial in combination with anethol and indole against *Candida albicans*. *J Agric Food Chem* 1993; 41: 1776-1779.
34. Shetty P, Atallah MT, Shetty K. Enhancement of total phenolic, L-DOPA and proline contents in germinating fava bean (*Vicia faba*) in response to bacterial elicitors. *Food Biotechnology* 2001; 15:47-67.
35. Randhir R, Shetty P, Shetty K. L-DOPA and total phenolic stimulation in dark germinated fava bean in response to peptide and phytochemical elicitors. *Process Biochemistry*, 2002; 37: 1247-1256.
36. Engleberger W, Hadding U, Etschenberg E, Graf E, Leyck S, Winkelmann J, Parnham MJ. Rosmarinic acid: A new inhibitor of complement C3 – convertase with anti-inflammatory activity. *Intl J Immunopharmac* 1988;10:729-737.
37. Kuhnt M, Probstle A, Rimpler H, Bauer A, Heinrich M. Biological and pharmacological activities and further constituents of *Hyptis verticillata*. *Planta Medica* 1995; 61: 227-232.
38. Fremont L. Biological effects of resveratrol. *Life Sciences* 2000; 66: 663-673.
39. Pinto MC, Garcia-Barrado JA, Macias P. Resveratrol is a potent inhibitor of the dioxygenase activity of lipoxygenase. *J Agric Food Chem* 1999; 47: 4842-4846.
40. Vatter DA, Shetty K. Solid-state production of phenolic antioxidants from cranberry pomace by *Rhizopus oligosporus*. *Food Biotechnology*, 2002; 16:189-210
41. Vatter DA, Shetty K. Ellagic acid production and phenolic antioxidant activity in cranberry pomace mediated by *Lentinus edodes* using solid-state system. *Process Biochemistry*, 2003; 39: 367-379.
42. Singh K, Khanna AK, Chander R. Protective effect of ellagic acid on t-butyl hydroperoxide induced lipid peroxidation in isolated rat hepatocytes. *Indian J Experimental Biology* 1999; 37: 939-943.
43. Narayanan BA, Re GG. IGF-II Down regulation associated cell cycle arrest in colon Cancer cells exposed to phenolic antioxidant ellagic acid. *Anticancer Res* 2001; 21: 359-364
44. Quiles JL, Mesa MD, Ramirez-Tortosa CL, Aguilera CM, Battino M, Ramirez-Tortosa MC. *Curcuma longa* extract supplementation reduces oxidative stress and attenuates aortic fatty streak development in rabbits. *Arterioscler Thromb Vasc Biol* 2002; 22: 1225-1231.
45. Dixon RA, Harrison MJ, Lamb CJ. Early events in the activation of plant defence responses. *Annl Rev of Phytopath* 1994; 32: 479-501.
46. Dixon RA, Paiva N. Stress-induced phenylpropanoid metabolism. *Plant Cell* 1995; 7: 1085-1097.
47. Rhodes JM, Wooltorton LSC. In: Kahl G, ed. *The biosynthesis of phenolic compounds in wounded plant storage tissues.. Biochemistry of wounded plant tissues*. Berlin: W de Gruyter, 1978; 286.
48. Brooker FL, Miller JE. Phenylpropanoid metabolism and phenolic composition of soybean [*Glycine max* (L) Merr.] leaves following exposure to ozone. *J Exp Bot* 1998; 49: 1191-1202.
49. Zimmerman YZ, Cohill PR. Heat shock and thermotolerance in plant and animal embryogenesis. *New Biology* 1991; 3: 641-650.
50. Yalpani N, Enyedi A J, Leon J, Raskin I. Ultraviolet light and ozone stimulate accumulation of salicylic acid, pathogenesis related proteins and virus resistance in tobacco. *Planta* 1994; 193: 372-376 .
51. Hahlbrock K, Scheel D. Physiology and molecular biology of phenylpropanoid metabolism. *Plant Mol Biol* 1989; 40: 347-369.
52. Graham TL. Flavanoid and isoflavanoid distribution in developing soybean seedling tissue and in seed and root exudates. *Plant Physiol* 1991; 95: 594-603.
53. Christie PJ, Alfenito MR, Walbot V. Impact of low temperature stress on general phenylpropanoid and anthocyanin pathways: Enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. *Planta* 1994; 194: 541-549.
54. Beggs CJ, Kuhn K, Bocker R, Wellmann E . Phytochrome induced flavanoid biosynthesis in mustard *Sinapsis alba* L) cotyledons: Enzymatic control and differential regulation of anthocyanin and quercetin formation. *Planta* 1987; 172: 121-126.
55. Lois R, Buchanan BB. Severe sensitivity to ultraviolet light radiation in *Arabidopsis* mutant deficient in flavanoid accumulation. Mechanisms of UV-resistance in *Arabidopsis*. *Planta* 1994; 194: 504-509.
56. Kurganova LN, Veselov AP, Goncharova TA, Sinitsyna YV. Lipid peroxidation and antioxidant system protection against heat shock in pea (*Pisum sativum* L.) Chloroplasts, *Fiziol. Rast. (Moscow)*, (Russ J Plant Physiol Engl Transl) 1997; 44: 725-730.
57. Retivin V, Opritov V, Fedulina SB. Generation of action potential induces preadaptation of *Cucurbita pepo* L. stem tissues to freezing injury. *Fiziol. Rast. (Moscow)*, (Russ. J. Plant Physiol., Engl. Transl.) 1997; 44: 499-510
58. Kuznetsov VV, Veststenko NV. Synthesis of heat shock proteins and their contribution to the survival of intact cucumber plants exposed to hyperthermia. *Fiziol. Rast. (Moscow)*, (Russ. J Plant Physiol Engl Transl) 1994; 41: 374-380.
59. Baraboi VA. Mechanisms of stress and lipid peroxidation. *Usp Sovr Biol* 1991; 11: 923-933.
60. Kurganova LN, Veselov AP, Sinitsina YV, Elikova EA. Lipid peroxidation products as possible mediators of heat stress response in plants. *Exp J Plant Physiology* 1999; 46: 181-185 .
61. Stewart CR, Larher F. Accumulation of amino acids and related compounds in relation to environmental stress. In: Mifflin BJ, ed. *The Biochemistry of Plants*, vol 5. New York: Academic Press, 1980; 609-635.
62. Thompson JF. Arginine synthesis, proline synthesis, and related process. In: Mifflin BJ, ed. *The Biochemistry of Plants*, vol 5. New York: Academic Press, 1980; 375-403.

63. Rhodes D. Metabolic Responses to stress. In: Davies DD, ed. Biochemistry of Plants vol 12. New York: Academic Press, 1987; 201-241.
64. Taylor CB. Proline and water deficit: ups and downs. The Plant Cell 1996; 8:1221-1224.
65. Hare PD, Cress WA. Metabolic implications of stress-induced proline accumulations in plants. Plant Growth Regulation 1997; 21: 79-102.
66. Briens M, Larher F. Osmoregulation in halophytic plants: a comparative study of soluble carbohydrates, polyols, betaines and free proline. Plant Cell and Environment 1982; 5: 287-292.
67. Jones MM, Osmond CB, Turner NC. Accumulation of solutes in leaves of sorghum sunflower in response to water deficits. Aust J Plant Physiol 1980; 7: 193-205.
68. Lansac AR, Sullivan CY, Johnson BE. Accumulation of free proline in sorghum (*Sorghum bicolor*) pollen. Can J Bot 1996; 74: 40-45.
69. Rhodes D, Handa S, Bressan RA. Metabolic changes associated with adaptation of plant cells to water stress. Plant Physiol 1986; 82: 890-902.
70. Sudhakar C, Reddy PS, Veeranjanyulu K. Effects of salt stress on enzymes of proline synthesis and oxidation in green gram (*Phaseolus aureus* Roxb.) seedlings. J Plant Physiol 1993; 141: 621-623.
71. LaRosa PC, Rhodes D, Rhodes JC, Bressan RA, Csonka LN. Elevated accumulation of proline in NaCl-adapted tobacco cells is not due to altered α -pyrroline-5-carboxylate reductase. Plant Physiol 1991; 96: 245-250.
72. Reddy PS, Veeranjanyulu K. Proline metabolism in senescing leaves of horsegram (*Macrotyloma uniflorum* Lam.). J Plant Physiol 1991; 137: 381-383.
73. Dallmier KA, Stewart CR. Effects of exogenous abscisic acid on proline dehydrogenase activity in maize (*Zea mays* L.). Plant Physiol 1992; 99: 762-764.
74. Elthon TE, Stewart CR. Effects of proline analogue L-thiazolidine-4-carboxylic acid on proline metabolism. Plant Physiol 1984; 74: 213-218.
75. Kiyosue T, Yoshida Y, Yamaguchi-Shinozaki K, Shinozaki K. A nuclear gene, encoding mitochondrial proline dehydrogenase, an enzyme involved in proline metabolism but downregulated by dehydration in *Arabidopsis*. The Plant Cell 1996; 8: 1323-1335.
76. Xin Z, Browse J. *Eskimo 1* mutants of *Arabidopsis* are constitutively freezing tolerant. Proc Natl Acad Sci USA 1998; 95: 7799-7804.
77. Kavi Kishore PB, Hong Z, Miao ZH, Hu CAA, Verma DPS. Overexpression of α -pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol 1995; 108: 1387-1394.
78. Martinez CA, Maestri M, Lani EG. In vitro salt tolerance and proline accumulation in Andean potato (*Solanum* spp.) differing frost resistance. Plant Science 1996; 116:177-184.
79. Paleg LG, Douglas TJ, van Daal A, Keech DB. Proline, betaine and other osmotic solutes protect enzymes against heat inactivation. Aust J Plant Physiol 1981; 8: 107-114.
80. Santoro MM, Liu Y, Khan SMA, Hou LX, Bolen DW. Increased thermal stability of Proteins in the presence of naturally occurring osmolytes. Biochemistry 1992; 31: 5278-5283.
81. Smirnov N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 1989; 28: 1057-1060.
82. Csonka L. Physiological and genetic responses of bacteria to osmotic stress. Microbiol Res 1989; 53: 121-147.
83. Csonka L, Hanson AD. Prokaryotic osmoregulation: genetics and physiology. Annu Rev Microbiol 1991; 45: 569-606.
84. Smith LT. Characterization of a gamma-glutamyl kinase from *Escherichia coli* that confers proline overproduction and osmotic tolerance. J Bacteriology 1985; 164:1088-1093.
85. Kohl DH, Schubert KR, Carter MB, Hagdorn CH, Shearer G. Proline metabolism in N_2 -fixing root nodules: energy transfer and regulation of purine synthesis. Proc Natl Acad Sci USA 1988; 85: 2036-2040.
86. Kohl DH, Lin JJ, Shearer G, Schubert KR. Activities of the pentose phosphate pathway and enzymes of proline metabolism in legume root nodules. Plant Physiol 1990; 94: 1258-1264.
87. Vartanian N, Hervochon P, Marcotte L, Larher F. Proline accumulation during drought rhizogenesis in *Brassica napus* var *oleifera*. J Plant Physiol 1992; 140: 623-628.
88. Skubatz H, Meeuse BJD, Bendich AJ. Oxidation of proline and glutamate by mitochondria of the inflorescence of Voodoo lily (*Sauromatum guttaum*). Plant Physiology 1989; 91: 530-535.
89. Venkamp JH, Koot JTM. The distribution of free amino acids, especially of proline, in the organs of field bean plants, *Vicia faba* L., during development in the field. J Plant Physiology 1984; 116: 343-349.
90. Singh BB, Gupta DP. Proline accumulation and relative water content in soybean (*Glycine max* L.) varieties under water stress. Ann Bot 1983; 52: 109-110.
91. Mehkri AA, Shashidhar VR, Uday Kumar M, Krishnasastri KS. Screening of varieties for relative drought tolerance in groundnut. Indian J Plant Physiol 1977; 20: 50-55.
92. Bouniols A, Margara J. Influence de l'adjonction d'acides amines au milieu de culture sur l'induction florale des bourgeons de *Cinchorium intybus* L. neofomes in vitro. CR Acad Ac Paris 1971; 273: 1193-1196.
93. Trigiano RN, Conger BV. Regulation of growth and somatic embryogenesis by proline and serine in suspension cultures of *Dactylis glomerata*. J Plant Physiology 1987; 130: 49-55.
94. Armstrong CL, Green CE. Establishment and maintenance of friable, embryogenic maize callus and the involvement of L-proline. Planta 1985; 164: 207-214.
95. Nuti-Ronchi V, Caligo MA, Nozzolini M, Luccarini G. Stimulation of carrot somatic embryogenesis by proline and serine. Plant Cell Reports 1984; 3: 210-214.
96. Shetty K, Asano Y. The influence of organic nitrogen sources on the induction of embryogenic callus in *Agrostis alba* L. J Plant Physiol 1991; 139:82-85.
97. Shetty K, Asano Y. Specific selection of embryogenic cell lines in *Agrostis alba* L using the proline analogue thioproline. Plant Science 1991; 79:259-263.
98. Shetty K, Shetty GA, Ezura H, Oosawa K. Stimulation of benzyladenine-induced *in vitro* shoot organogenesis from cotyledons of *Cucumis sativa* L by proline and abscisic acid. Plant Tissue Culture Lett 1992; 9:104-108
99. Shetty K, Shetty GA, Nakazaki Y, Yoshioka K, Asano Y, Oosawa K. Stimulation of benzyladenine-induced *in vitro* shoot organogenesis in *Cucumis melo* L by proline, salicylic acid and aspirin. Plant Science 1992; 84:193-199.
100. Shetty K, McKersie BD. Proline, thioproline and potassium mediated stimulation of somatic embryogenesis in alfalfa (*Medicago sativa* L). Plant Science 1993; 88:185-193.
101. Milazzo MC, Kellet G, Haynesworth K, Shetty K. Regulation of benzyladenine-induced *in vitro* shoot organogenesis and endogenous proline in melon (*Cucumis melo* L.) by exogenous proline, ornithine and proline analogues. J Agric Food Chem 1998; 46:2402-2406.

102. Milazzo MC, Zheng Z, Kellet G, Haynesworth K, Shetty K. Stimulation of benzyladenine-induced in vitro shoot organogenesis and endogenous proline in melon (*Cucumis melo* L.) by fish protein hydrolysates in combination with proline analogues. *J Agric Food Chem* 1999;47:1771-1775.
103. Phang JM. The regulatory functions of proline and pyrroline-5-carboxylic acid. *Curr Topics in Cell Regulation* 1985; 25: 91-132.
104. Hagedorn CH, Phang JM. Transfer of reducing equivalents into mitochondria by the interconversions of proline and α -pyrroline-5-carboxylate. *Arch Biochem Biophys* 1983; 225: 95-101.
105. Rayapati JP, Stewart CR. Solubilization of a proline dehydrogenase from maize (*Zea mays* L.) mitochondria. *Plant Physiol* 1991; 95: 787-791.
106. Yeh, GC, Phang JM. The function of pyrroline-5-carboxylate reductase in human erythrocytes. *Biochem Biophys Res Commun* 1980; 94: 450-457.
107. Phang JM, Downing SJ, Yeh GC, Smith RJ, Williams JA. Stimulation of hexosemonophosphate-pentose pathway by α -pyrroline-5-carboxylic acid in human fibroblasts. *Biochem Biophys Res Commun* 1979; 87: 363-370.
108. Hagedorn CH, Phang, JM. Catalytic transfer of hydride ions from NADPH to oxygen by the interconversions of proline and α -pyrroline-5-carboxylate. *Arch Biochem Biophys* 1986; 248: 166-174.
109. Kwok D, Shetty K. Effect of proline and proline analogues on total phenolic and rosmarinic acid levels in shoot clones of thyme (*Thymus vulgaris* L.). *J Food Biochemistry*, 1998; 22:37-51.
110. Yang R, Shetty K. Stimulation of rosmarinic acid in shoot cultures of oregano (*Origanum vulgare*) clonal line in response to proline, proline analogue and proline precursors. *J Agric Food Chem* 1998; 46:2888-2893.
111. Bela J, Shetty K. Somatic embryogenesis in anise (*Pimpinella anisum* L.): The effect of proline on embryogenic callus formation and ABA on advanced embryo development. *J Food Biochemistry* 1999; 23:17-32.
112. Perry PL, Shetty K. A model for involvement of proline during *Pseudomonas*-mediated stimulation of rosmarinic acid. *Food Biotechnology* 1999; 13:137-154.
113. Lendzian KJ. Modulation of glucose-6-phosphate dehydrogenase by NADPH, NADP⁺ and dithiothreitol at variable NADPH/NADP⁺ ratios in an illuminated reconstituted spinach (*Spinacia oleracea* L.) chloroplast system. *Planta* 1980; 148: 1-6.
114. Copeland L, Turner JF. The regulation of glycolysis and the pentose-phosphate pathway. In: Stumpf P and Conn EE, eds. *The Biochemistry of Plants*, Vol. 11. New York: Academic Press 1987; 107-125.
115. Jost A, Perlman S, Valentino O, Castinier M, Scholler R, Magre S. Experimental control of the differentiation of Leydig cells in the rat fetal testis. *Proc Natl Acad Sci USA* 1988; 85: 8094-8097.
116. Al-Amier H, Mansour BMM, Toaima N, Korus RA, Shetty K. Tissue culture-based screening for selection of high biomass and phenolic-producing clonal lines of Lavender using *Pseudomonas* and azetidine-2--carboxylate. *J Agric Food Chem* 1999; 47:2937-2943.
117. Al-Amier H, Mansour BMM, Toaima N, Korus RA, Shetty, K. Screening of high biomass and phenolic-producing clonal lines of Spearmint in tissue culture using *Pseudomonas* and azetidine-2-carboxylate. *Food Biotechnology* 1999; 13:227-253.
118. Al Amier HA, Mansour, BMM, Toaima N, Craker L, Shetty K. Tissue culture for phenolics and rosmarinic acid in thyme. *J Herbs Spices & Medicinal Plants* 2001; 8:31-42.
119. Eguchi Y, Curtis OF, Shetty K. Interaction of hyperhydricity-preventing *Pseudomonas* spp. with oregano (*Origanum vulgare*) and selection of high rosmarinic acid-producing clones. *Food Biotechnology* 1996; 10:191-202.
120. Shetty K, Carpenter TL, Kwok D, Curtis OF, Potter TL. Selection of high phenolics-containing clones of thyme (*Thymus vulgaris* L.) using *Pseudomonas* spp. *J Agric Food Chem* 1996; 44:3408-3411.
121. Yang R, Curtis OF, Shetty K. Selection of high rosmarinic acid-producing clonal lines of rosemary (*Rosmarinus officinalis*) via tissue culture using *Pseudomonas* sp. *Food Biotechnology* 1997; 11:73-88.
122. McCue P, Zheng Z, Pinkham JL, Shetty K. A model for enhanced pea seedling vigour following low pH and salicylic acid treatments. *Process Biochemistry* 2000; 35: 603-613.
123. Andarwulan N, Shetty K. Improvement of pea (*Pisum sativum*) seed vigour by fish protein hydrolysates in combination with acetyl salicylic acid. *Process Biochemistry* 1999; 35: 159-165.
124. Duval B, Shetty K. The stimulation of phenolics and antioxidant activity in pea (*Pisum sativum*) elicited by genetically transformed anise root extract. *J Food Biochemistry* 2001; 25:361-377.
125. McCue P, Shetty K. A biochemical analysis of mungbean (*Vigna radiata*) response to microbial polysaccharides and potential phenolic-enhancing effects for nutraceutical applications. *Food Biotechnology* 2002; 6:57-79.
126. McCue P, Shetty K. Clonal herbal extracts as elicitors of phenolic synthesis in dark-germinated mungbeans for improving nutritional value with implications for food safety. *J Food Biochemistry* 2002; 26: 209-232.
127. Shetty P, Atallah MT, Shetty K. Effects of UV treatment on the proline-linked pentose phosphate pathway for phenolics and L-DOPA synthesis in dark germinated *Vicia faba*. *Process Biochemistry* 2002; 37: 1285-1295.
128. Shetty P, Atallah MT, Shetty K. Stimulation of total phenolics, L-DOPA and antioxidant activity through proline-linked pentose phosphate pathway in response to proline and its analogue in germinating fava beans (*Vicia faba*) *Process Biochemistry* 2003; 38: 1707-1717.
129. Randhir R, Shetty K. Microwave-induced stimulation of L-DOPA, phenolics and antioxidant activity in fava bean (*Vicia faba*) for Parkinson's diet. *Process Biochemistry* 2003; In press.
130. Randhir R, Shetty K. (2003) Light-mediated fava bean (*Vicia faba*) response to phytochemical and protein elicitors and consequences on nutraceutical enhancement and seed vigour. *Process Biochemistry*, 38: 945-952.
131. McCue, Shetty K. A role for amylase and peroxidase-linked polymerization in phenolic antioxidant mobilization in dark-germinated soybean and implications for health. *Process Biochemistry* 2003; In press.
132. Zheng Z, Shetty K. Effect of apple pomace-based *Trichoderma* inoculants on seedling vigour in pea (*Pisum sativum*) germinated in potting soil. *Process Biochemistry* 1999; 34:731-735.
133. Zheng Z, Shetty K. Enhancement of pea (*Pisum sativum*) seedling vigour and associated phenolic content by extracts of apple pomace fermented with *Trichoderma* spp. *Process Biochemistry* 2000; 36:79-84.

134. Shetty K, Curtis OF, Levin RE, Witkowsky R, Ang W. Prevention of vitrification associated with *in vitro* shoot culture of oregano (*Origanum vulgare*) by *Pseudomonas* spp. J Plant Physiol 1995; 147:447-451.
135. Shetty K, Curtis OF, Levin RE. Specific interaction of mucoid strains of *Pseudomonas* spp. with oregano (*Origanum vulgare*) clones and the relationship to prevention of hyperhydricity in tissue culture. J Plant Physiol 1996; 149:605-611.
136. Ueno K, Shetty K. Prevention of hyperhydricity in oregano shoot cultures is sustained through multiple subcultures by selected polysaccharide-producing soil bacteria without re-inoculation. Appl Microbiol Biotechnology 1998; 50:119-124.
137. Strycharz S, Shetty K. Effect of *Agrobacterium rhizogenes* on phenolic content of *Mentha pulegium* elite clonal line for phytoremediation applications. Process Biochemistry, 2002a; 38: 287-293.
138. Zheng Z, Pinkham JL, Shetty K. Identification of polymeric dye-tolerant oregano (*Origanum vulgare*) clonal lines by quantifying total phenolics and peroxidase activity. J Agric Food Chem 1998; 46:4441-4446.
139. Zheng Z, Shetty K. Azo dye-mediated regulation of total phenolics and peroxidase activity in thyme (*Thymus vulgaris* L.) and rosemary (*Rosmarinus officinalis* L.) clonal lines. J Agric Food Chem 2000; 48:932-937.
140. Strycharz S, Shetty K. Peroxidase activity and phenolic content in elite clonal lines of *Mentha pulegium* in response to polymeric dye R-478 and *Agrobacterium rhizogenes*. Process Biochemistry 2002; 37: 805-812
141. Strycharz S, Shetty K. Response of oregano (*Origanum vulgare*) clonal lines to *Pseudomonas* sp. Z strain and polydye R-478 and implications for hyperhydricity prevention in tissue culture. Process Biochemistry 2002; 38: 343-350.
142. Zheng Z, Sheth U, Nadiga M, Pinkham JL, Shetty K. A model for the role of proline-linked phenolic synthesis and peroxidase activity associated with polymeric dye tolerance in oregano. Process Biochemistry 2001; 36: 941-946.
143. Andarwulan N, Fardiaz D, Wattimena GA, Shetty K. Antioxidant activity associated with lipid and phenolic mobilization during seed germination of *Pangium edule* Reinw. J Agric Food Chem 1999; 47:3158-3163.
144. Andarwulan N, Shetty K. Phenolic synthesis in differentiated tissue cultures of untransformed and *Agrobacterium*-transformed roots of anise (*Pimpinella anisum* L.). J Agric Food Chem 1999; 47:1776-1780.
145. Andarwulan N, Shetty K. Stimulation of novel phenolic metabolite, epoxy-Pseudoisoeugenol-(2-Methylbutyrate) [EPB], in transformed anise (*Pimpinella anisum* L.) root cultures by fish protein hydrolysates. Food Biotechnology 2000; 14:1-20.
146. Duval B, Shetty K, Thomas WH. Phenolic compounds and antioxidant properties in the snow alga *Chlamydomonas nivalis* after exposure to UV light. J Applied Phycology 1999; 11:559-566.
147. Zheng Z, Shetty K. Cranberry processing waste for solid-state fungal inoculant production. Process Biochemistry 1998; 33:323-329.
148. Zheng Z, Shetty K. Solid state production of beneficial fungi on apple processing waste using glucosamine as the indicator of growth. J Agric Food Chem 1998; 46:783-787.
149. Zheng Z, Shetty K. Solid-state bioconversion of phenolics from cranberry pomace and role of *Lentinus edodes* beta-glucosidase. J Agric Food Chem 2000; 48:895-900.
150. Zheng Z, Shetty K. Solid-state production of polygalacturonase by *Lentinus edodes* using fruit processing wastes. Process Biochemistry 2000; 35:825-830.
151. McCue P, Shetty K. Role of carbohydrate - cleaving enzymes in phenolic antioxidant mobilization from whole soybean fermented with *Rhizopus oligosporus*. Food Biotechnology 2003; 17:27-37.
152. Eguchi Y, Bela JS, Shetty K. Stimulation of somatic embryogenesis in anise (*Pimpinella anisum*) by fish protein hydrolysates in combination with proline. J Herbs Spices and Medicinal Plants 1997;5: 61- 68.
153. Eguchi Y, Milazzo MC, Ueno K, Shetty K. Partial improvement of vitrification and acclimation of oregano (*Origanum vulgare*) tissue cultures by fish protein hydrolysates. J Herbs, Spices and Medicinal Plants 1999; 6: 29-38.
154. Andarwulan N, Shetty K. Influence of fish protein hydrolysates in combination with acetyl salicylic acid on hyperhydricity reduction and phenolic synthesis in oregano (*Origanum vulgare*) tissue cultures. J Food Biochemistry 1999; 23:619-635.
155. Rao MV, Paliyath G, Ormrod DP. Ultraviolet-B and Ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. Plant Physiology 1996; 110: 125-136.
156. Lewis NG. Plant Phenolics. In: Alscher RG, Hess JL, eds. Antioxidants in higher plants. Boca Raton, FL: CRC Press 1993; 135-169.
157. Bowler C, Van Camp W, Van Montagu M, Inze D. Superoxide dismutase in plants. CRC Crit Rev Plant Sci 1994; 199-218.
158. Creissen GP, Edwards EA, Mullineaux PM. Glutathione reductase and ascorbate peroxidase. In: Foyer CH, Mullineaux PM, eds. Causes of photooxidative stress and amelioration of defence systems in plants. Boca Raton FL: CRC Press, 1994; 343-364.
159. Pinhero RG, Rao MV, Paliyath G, Murr DP, Fletcher RA. Changes in activities of antioxidant enzymes and their seedling relationship to genetic and Paclobutrazol-induced chilling tolerance of maize seedlings. Plant Physiology 1997; 114: 695-704.
160. Foyer CH, Descourvieres P, Kunert KJ. Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. Plant Cell Environ 1994; 17: 507-523.
161. Gasper TH, Penel C, Hagega D, Greppin H. Peroxidases in plant growth, differentiation and development processes. In: Lobarzewski J, Greppin H, Penel C, Gasper TH, eds. Biochemical, Molecular and Physiological Aspects of Plant Peroxidases.. University de Geneve, Switzerland, 1991; 249-280.
162. Chen GX, Asada K. Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant Cell Physiology 1989; 30: 987-998.
163. Otter T, Polle A. The influence of apoplastic ascorbate on the activities of the cell-wall associated peroxidases and NADH-oxidases in needles of Norway spruce (*Picea abies* L.). Plant Cell Physiology 1994; 35: 1231-1238.
164. Li J, Ou-Lee TM, Raba R, Amundson RG, Last RL. *Arabidopsis* flavonoid mutants are hypersensitive to ultraviolet-B radiation. Plant Cell 1993; 5: 171-179.
165. Kowaloff EM, Granger AS, Phang JM. Alterations in proline metabolic enzymes with mammalian development. Metabolism 1976; 25: 1087-1094.
166. Phang JM, Downing SJ, Yeh GC, Smith RJ, Williams JA, Hagedorn CH. Stimulation of the hexosemonophosphate-pentose pathway by pyrroline-5-carboxylate in cultured cells. J Cell Physiol 1982; 110; 255-261.

167. Yeh GC, Phang JM. Pyrroline-5-carboxylate stimulates the conversion of purine antimetabolites to their nucleotide forms by a redox-dependent mechanism. *J Biol Chem* 1983; 258: 9774-9779.
168. Yeh GC, Phang JM. Stimulation of phosphoribosyl pyrophosphate and purine nucleotide production by pyrroline-5-carboxylate in human erythrocytes. *J Biol Chem* 1988; 263: 13083-13089.
169. Donald SP, Sun, X-Y, Chien-An A, Yu J, Mei, JM, Valle D, Phang JM. Proline oxidase, encoded by p53-induced gene-6, catalyzes the generation of proline-dependent reactive oxygen species. *Cancer Research* 2001; 61:1810-1815.
170. Lin WW, Hu CA, Valle D. Cloning, characterization and expression of cDNAs encoding human delta1-pyrroline-5-carboxylate dehydrogenase. *J Biol Chem* 1996; 271: 9795-9800.
171. Campbell HD, Webb GC, Young IG. A human homologue of *Drosophila melanogaster* sluggish-A (proline oxidase) gene maps to 22q11.2, and is a candidate for type I hyperprolinaemia. *Human Genet* 1997; 101: 69-74.
172. Maxwell SA, Davis GE. Differential gene expression in p53-mediated apoptosis-resistant vs. apoptosis-sensitive tumor cell lines. *Proc Natl Acad Sci USA* 2000; 97: 13009-13014.
173. Maxwell SA, Rivera A. Proline oxidase induces apoptosis in tumor cells, and its expression is frequently absent or reduced in renal carcinomas. *J Biol Chem* 2003; 278: 9784-9789.
174. Liu, H. et al. Genetic variation at the 22q11 PRODH2/DGCR6 locus presents unusual pattern and increases susceptibility to schizophrenia. *Proc Natl Acad Sci USA* 2002; 99: 3717-3722.
175. Droge W. Free radicals in the physiological control of cell function. *Physiological Reviews* 2002; 82: 47-95.
176. Dreher D, Junod AF. Role of oxygen free radicals in cancer development. *Eur J Cancer* 1996; 32A: 30-38.
177. Ha HC, Thiagalingam A, Nelkin BD, Casero RA Jr. Reactive oxygen species are critical for the growth and differentiation of medullary thyroid carcinoma cells. *Clin Cancer Res* 2000; 6: 3783-3787.
178. Alexander RW. Theodore Cooper Memorial Lecture. Hypertension and the pathogenesis of atherosclerosis: oxidative stress and the mediation of arterial inflammatory response: a new perspective. *Hypertension* 1995; 25: 155-161.
179. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 1994; 74: 1141-1148.
180. Araujo V, Arnal C, Boronat M, Ruiz E, Dominguez C. Oxidant-antioxidant imbalance in blood of children with juvenile rheumatoid arthritis. *Biofactors* 1998; 8: 155-159.
181. Mapp PI, Grootveld MC, Blake DR. Hypoxia, oxidative stress and rheumatoid arthritis. *Br Med Bull* 1995; 51: 419-436.
182. Multhaup G, Ruppert T, Schlicksupp A, Hesse L, Behr D, Masters CL, Beyreuther K. Reactive oxygen species in Alzheimer's disease. *Biochem Pharmacol* 1997; 54: 533-539.
183. Wolff SP. Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. *Br Med Bull* 1993; 49: 642-652.
184. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991; 40: 405-412.
185. De Mattia G, Bravi MC, Laurenti O, Cassone-Faldetta M, Armeinto A, Ferri C, Balsano F. Influence of reduced glutathione infusion on glucose metabolism in patients with non-insulin-dependent diabetes mellitus. *Metabolism* 1998; 47: 993-997.
186. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycemic damage. *Nature* 2000; 404: 787-790.
187. Van Dam PS, van Asbeck BS, Erkelens DW, Marx JJM, Gispen WH, Bravenboer B. The role of oxidative stress in neuropathy and other diabetic complication. *Diabetes Metab Rev* 1995; 11: 181-192.
188. Labriola D, Livingston R. Possible interactions between dietary antioxidants and chemotherapy. *Oncology* 1999; 13: 1003-1008.
189. Freudenheim JL, Marshall JR, Vena JE. Premenopausal breast cancer risk and intake of vegetables, fruits and related nutrients. *J Natl Cancer Inst* 1996; 88: 340-348.
190. Morel Y, Barouki R. Repression of gene expression by oxidative stress. *Biochem J* 1999; 342: 481-496.
191. Margulis L. Symbiosis everywhere and other chapters. In : *Symbiotic Planet (A new view of Evolution)*. Basic Books (A Member of the Perseus Books Group). New York, 1998.
192. Cowan MM. Plant products as antimicrobial agents. *Clinical Microbiology Reviews* 1999; 12: 564-582.
193. Beuchat LR. Antimicrobial properties of spices and their essential oils. In: Board RG, Dillon V, eds. *Natural Antimicrobial systems in food preservation*. Wallingford, Oxon, UK: CAB International, 1994; 167-179.
194. Shelef LA. Antimicrobial effects of spices. *J Food Safety* 1986; 29-44.
195. Walker JRL. Antimicrobial compounds in food plants. In: Board RG, Dillon V, eds. *Natural Antimicrobial systems in food preservation*. Wallingford, Oxon, UK: CAB International, 1994; 181-204.
196. Stern JL, Hagerman AE, Steinberg PD and Mason PK. Phlorotannin-protein interactions. *J Chem Ecol* 1996; 22: 1887-1899.
197. Seaberg A, Labbe RL, Shetty K. Inhibition of *Listeria monocytogenes* in broth and meat by clonal extracts of oregano. *Food Biotechnology* 2003; 17:129-149.
198. Beuchat LR. Sensitivity of *Vibrio parahaemolyticus* to spices and organic acids. *J Food Sci* 1976; 41; 899-902.
199. Aktug SE, Karapinar M. Sensitivity of some common food poisoning bacteria to thyme, mint and bay leaves. *Int J Food Microbiology* 1986; 3: 349-354.
200. Shelef LA, Jyothi EK, Bugarelli MA. Growth of enteropathogenic and spoilage bacteria in sage-containing broth and foods. *J Food Sci* 1984; 49: 737-740.
201. Farbood MI, McNeil JH, Ostovar K. Effect of rosemary spice extractive on the growth of microorganisms in meat. *J Milk Food Technol* 1976; 39: 675-679.
202. Herald PJ, Davidson PM. Antibacterial activity of selected hydroxycinnamic acids. *J Food Sci* 1983; 48: 1378-1379.
203. Chung KT, Murdock CA. Natural systems for preventing contamination and growth of microorganisms in foods. *Food Microstruct* 1991; 10: 361-366.

204. Scalbert A. Antimicrobial properties of tannins. *Phytochemistry* 1991; 30: 3875-3883.
205. Urs NVRR, Dunleavy JM. Enhancement of the bactericidal activity of peroxidase system by phenolic compounds. *Phytopathology* 1975; 65: 686-690.
206. Conner DE, Beuchat LR, Worthington RE, Kautter DA. Effects of essential oils and oleoresins of plants on ethanol production, respiration and sporulation of yeasts. *Int J Food Microbiol* 1984; 1: 63-74.
207. Baranowski JD, Davidson PM, Nagel CW, Branen AL. Inhibition of *Saccharomyces cerevisiae* by naturally occurring hydroxycinnamates. *J Food Sci* 1980; 45: 592-594.
208. Mason TL, Wasserman BP. Inactivation of red beet beta-glucan synthase by native and oxidized phenolic compounds. *Phytochemistry* 1987; 26: 2197-2202.