Perspectives in Nutrigenomics and Nutrigenetics

Michael Fenech
Director, Personalised Nutrition and Genome Health Laboratory, CSIRO Food and Nutrition, Adelaide, Australia

Introduction

The efficacy and safety of nutritional intervention is dependent on a thorough understanding of **(1)** which nutrients may be deficient or in excess in a population, **(2)** the pathologies induced by specific nutritional imbalances at the genomic, transcriptomic, proteomic and metabolic levels, and **(3)** appropriate diagnostic tools to monitor outcomes at the population and genetic subgroup level. The emerging sciences of nutrigenomics and nutrigenetics which address these issues are expected to contribute substantially to the elimination of malnutrition and to the optimizing of health outcomes to a greater extent than would otherwise be possible by using conventional approaches alone.

Definitions of nutrigenetics and nutrigenomics

Nutrigenetics is the science of the effect of genetic variation on cellular and organism response to dietary intervention. In contrast, the aim of **nutrigenomics** science is to understand how nutrients and bioactive food compounds affect gene expression and maintenance of genome integrity. Exploitation of this genomic information, along with high-throughput "omic" technologies, allows the acquisition of new knowledge aimed at obtaining a better understanding of nutrient-gene interactions depending on genotype, with the ultimate goal of developing nutrition strategies for optimal health and disease prevention at the individual, genetic subgroup and population level.

Therefore, the fundamental hypotheses underpinning the science of nutrigenetics and nutrigenomics are the following:

- Nutrition may exert its impact on health outcomes by directly affecting expression of genes in critical metabolic pathways and/or indirectly by affecting the incidence of genetic mutation at the base sequence or chromosomal level, which in turn causes alterations in gene dosage and gene expression.
- The health effects of nutrients and nutriomes (nutrient combinations) depend on inherited genetic variants that alter the uptake and metabolism of nutrients and/or the molecular interaction of enzymes with their nutrient cofactor or metabolites and hence the activity of biochemical reactions.
- Better health outcomes can be achieved if nutritional requirements are customized for each individual or genetic subgroup, taking into consideration both inherited and acquired genetic characteristics depending on life stage, dietary preferences and health status.

Perspective on potential implications for nutrition

A nutrigenetic and nutrigenomic approach has important consequences to the way public health strategies aimed at nutrition are designed and implemented. For example, dietary reference values (e.g., recommended dietary allowance or safe upper limits) are designed for the general population and not optimized for genetic subgroups which may differ critically in the activity of transport proteins for a micronutrient and/or enzymes that require that micronutrient as a cofactor. The ultimate goal is to **(1)** match the nutriome (i.e., nutrient intake combination) with the genome profile so that DNA integrity, gene expression, metabolism and cell function can occur normally and in a homeostatically sustainable manner, and **(2)** provide better mechanistic interpretation of data from epidemiological and clinical intervention studies regarding health impacts of dietary factors that may help to refine recommendations so that they can also be specifically targeted to individuals and genetic subgroups.

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TABLE 1: Examples of common polymorphisms in genes that affect vitamin transport / metabolism and / or function of an enzyme for which the micronutrient is a cofactor

Micronutrient	Gene	Function of protein or enzyme	Polymorphism	Molecular or health effect of polymorphism
Vitamin A	BCMO1	Cleaves β-carotene into two molecules of all-*trans*-retinal	R267S: rs12934922; A379V: rs7501331	A β-carotene supplementation study with healthy female volunteers indicated that carriers of the 379V and 267S/379V variant alleles had 160% and 240% higher fasting β-carotene concentrations compared to wild-type carriers. More importantly, the conversion efficiency of BCMO1 for β-carotene was 32% reduced in carriers of the 379V variant allele and 69% reduced in carriers of the 267S/379V variant allele compared to the wildtype.[12,13]
Folate	MTHFR	Converts 5,10-methylene-tetrahydrofolate to 5-methyl-tetrahydrofolate (5-MTHF)	C677T: rs1801133	The C677T polymorphism of MTHFR reduces the activity of the enzyme by 50% in TT homozygotes and increases the risk of hyperhomocysteinemia, a risk factor for pregnancy complications, cardiovascular disease and dementia.[9–11]
Vitamin B_{12}	MTR	Converts 5-MTHF and homocysteine to methionine and tetrahydrofolate. B_{12} is the cofactor for MTR	A2756G: rs1805087	Although the functional effect of this polymorphism methionine synthase has not been established, some studies have shown a relatively higher concentration of plasma homocysteine among individuals with the AA genotype. The AA genotype is also associated with more chromosomal DNA damage and reduced disease-free longevity.[14–15]
Vitamin C	SLC23A1	SVCT1, encoded by the gene *SLC23A1*, is predominantly responsible for high capacity vitamin C transport across membranes	rs6596473 rs11950646	Skibola et al observed an increased risk of follicular lymphoma associated with two SNPs (rs6596473 and rs11950646), one of which (rs6596473) is associated with decreased plasma ascorbate levels.[16,17]
Vitamin D	GC	GC codes for the vitamin D binding protein which transports vitamin D throughout the body	rs2282679	rs2282679 within the GC gene is significantly associated with concentrations of GC protein with the minor allele associated with lower levels of vitamin D in plasma.[18]
Vitamin E	ABCA1	The *ABCA1* gene belongs to a group of genes called the ATP-binding cassette family, which provides instructions for making proteins that transport molecules across cell membranes	rs11789603 rs2274873	rs11789603 and rs2274873 polymorphisms predict the plasma chylomicron α-tocopherol level of a subject in response to an α-tocopherol-rich meal.[19,20]

We now live in an era when it is becoming increasingly affordable to have one's genome determined, providing information on a wide spectrum of critical mutations (e.g., single-nucleotide mutation, insertions-deletions, block substitutions, inversions or copy number variants) in critical genes involved in nutrient metabolism and /or pathways requiring micronutrients as cofactors.[1] Gender itself is a critical genetic variation that affects micronutrient requirements for health maintenance.[2] The key challenge is to determine whether it is possible to utilize this information meaningfully to provide reliable and predictable personalized dietary recommendations for specific health outcomes.

An important emerging aspect of nutrient-gene interaction studies with the potential for both intra- and trans-generational effects is epigenetics.[3] Epigenetics refers to the processes that regulate how and when certain genes are turned on and off, while epigenomics pertains to analysis of epigenetic changes in

a cell or entire organism. Epigenetic processes have a strong influence on normal growth and development, and this process is deregulated in diseases such as cancer. Diet on its own, or by interaction with other environmental factors, can cause epigenetic changes that may turn certain genes on or off. As a result, epigenetic silencing of genes that would normally protect against a disease could make people more susceptible to developing that disease later in life. The epigenome which may be to a limited extent heritable and modifiable by diet is the pattern of global and gene-specific DNA methylation, histone modifications and chromatin-associated proteins which control expression of housekeeping genes and suppress the expression of parasitic DNA such as transposons (jumping genes).[4]

Nutrient-gene interactions

About 70% of enzymes require minerals or vitamins as cofactors for their function or, for example in the case of zinc finger proteins, the mineral forms an integral part of its structure.[5] Therefore, even in the absence of loss of function gene mutations, deficiency in the cofactor will cause a transport protein or enzyme to malfunction. The knock-on effects of such deficiency may spread to affect other genes. For example, zinc is required for the function of the DNA repair protein OGG1 which repairs oxidized guanine in DNA, but its malfunction will result in accumulation of 8-oxoguanine, which leads to point mutations or down-regulation of gene expression if CpG islands are affected.[6,7] The latter was shown to be an important cause of changes in gene

FIGURE 1: DNA damage biomarkers that have been successfully used to investigate the effect of nutritional deficiency or excess on genome integrity [22]

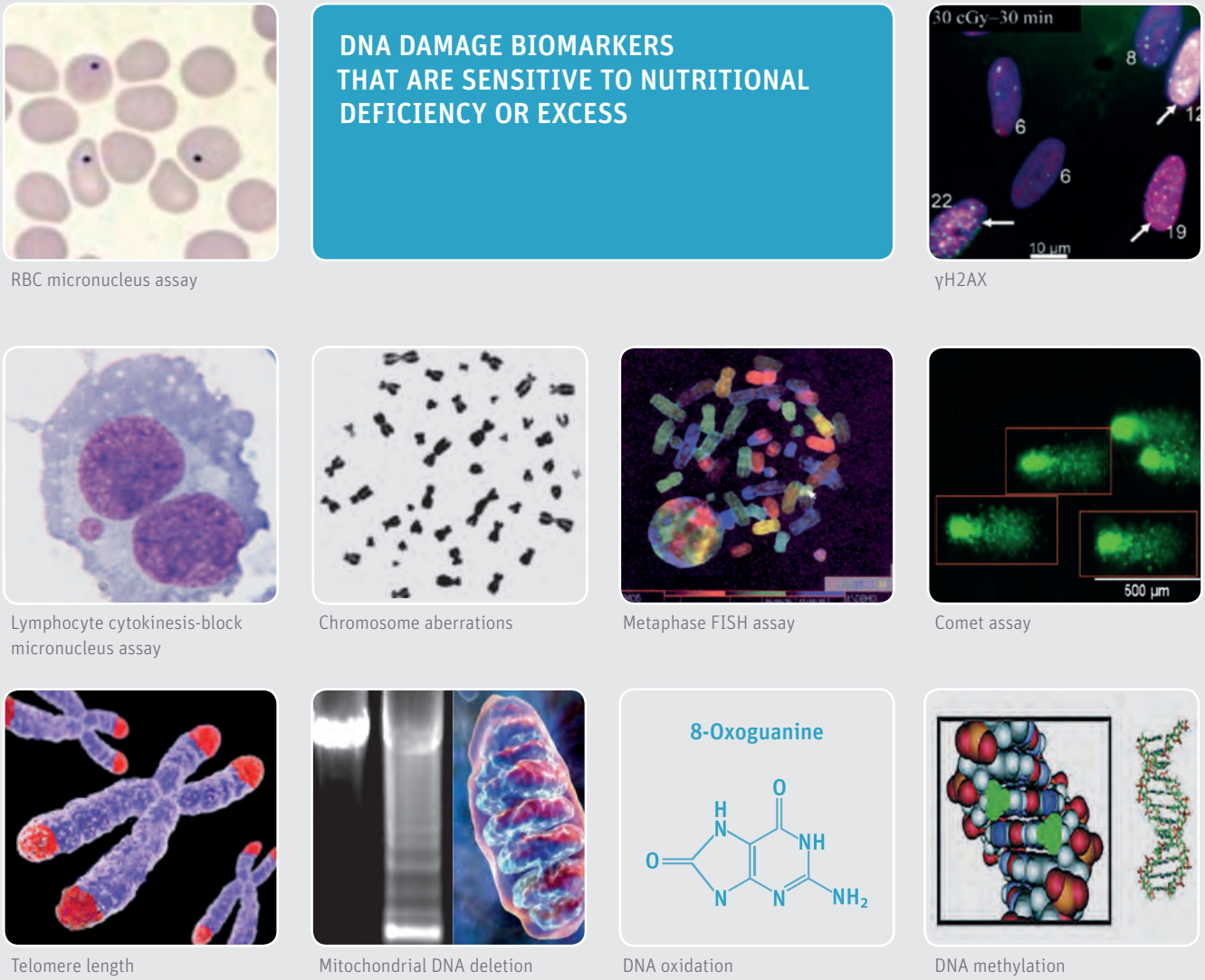
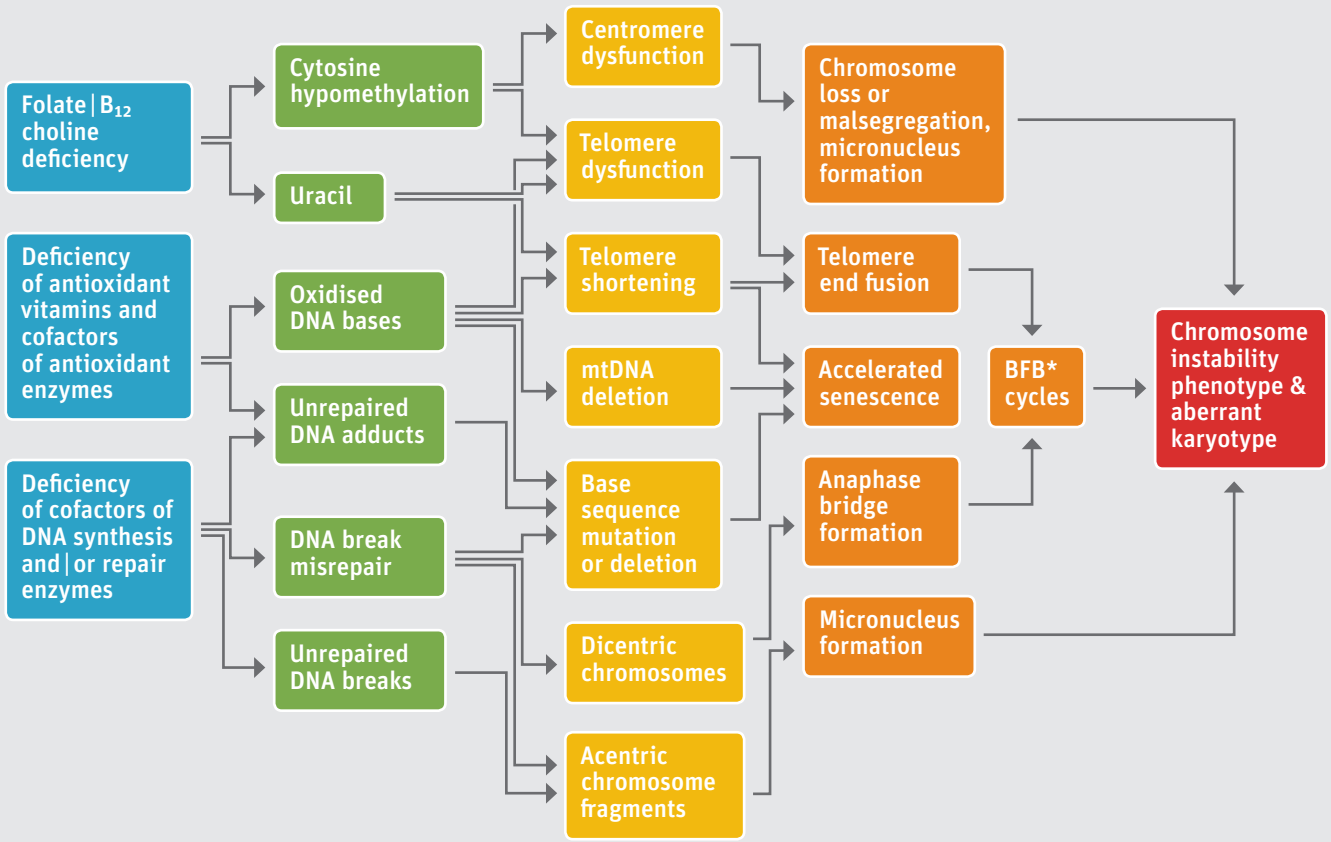


FIGURE 2: Possible mechanisms by which micronutrient deficiencies could cause damage to the genome [22]



***BFB** = breakage-fusion-bridge cycles.

expression with age in the human brain.[8] Another complex case is that of one-carbon metabolism in which folate is converted to various forms to play its role as a methyl donor in pivotal reactions such as the synthesis of thymidine from uridine required for DNA synthesis or repair and the conversion of homocysteine to methionine, which ultimately drives cell proliferation and maintenance of DNA methylation and gene regulation after its conversion to the methyl donor S-adenosylmethionine.[9]

Common polymorphisms affect nutritional requirements

For each micronutrient, there exist common polymorphisms in key genes that limit its transport, its metabolism to an active form, and the ability of tissues to store and/or mobilize it when needed. Some of these common polymorphisms and their effects are listed in **Table 1**. One that has been most investigated in thousands of epidemiological and intervention studies is the C677T polymorphism in the methylene-tetrahydrofolate reductase (MTHFR) gene, which requires riboflavin (vitamin B_2) as cofactor and is essential to convert 5,10-methylene-tetrahydrofolate (5,10-MTHF) to 5-methyl-tetrahydrofolate (5-MTHF), the form of folate that is transported in the blood and is required to convert homocysteine to methionine.[10] Interactive effects between the C677T polymorphism, 5,10-MTHF and vitamin B_2 ultimately determine the bioavailability of folate for DNA or methionine synthesis, affecting chromosomal stability, reproductive outcomes and risk for degenerative diseases of old age.[9–11] Other examples of common polymorphisms in genes that affect bioavailablity and bioefficacy of vitamin A, folate, vitamins B_{12}, C, D and E are given in **Table 1**.

DNA damage prevention and dietary reference values

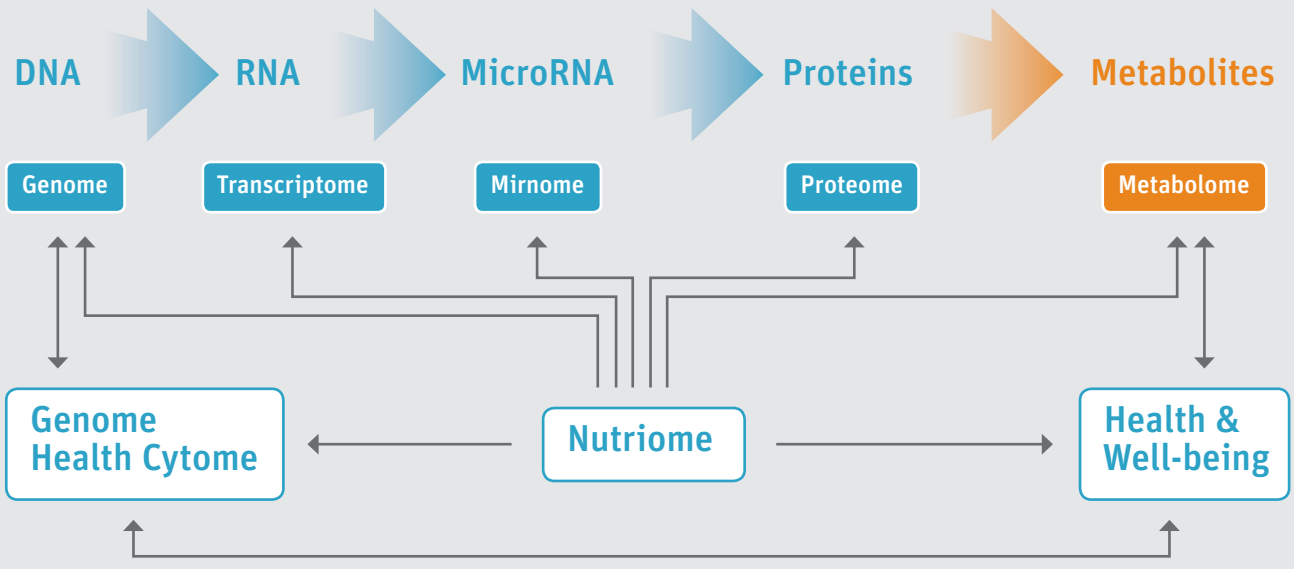
Damage to the genome is the most fundamental pathology which can be measured at the molecular, chromosomal or cytological level (**Figure 1**).[21] The capacity of cells to replicate their genetic material accurately is critical for all stages of life, including fertility, conception, development during the first 1000 days of life, growth during childhood, intellectual development, immune function and tissue regeneration during adulthood, and

prevention of cancer and accelerated ageing. Several micronutrients play an important role in protecting against DNA damage events, induced by endogenous and exogenous factors, by acting as cofactors or substrates for enzymes that detoxify genotoxins as well as enzymes involved in DNA repair, methylation, and synthesis (**Figure 2**).[22] In addition, it is evident that either micronutrient deficiency or micronutrient excess can modify genome stability and that these effects may also depend on nutrient-nutrient and nutrient-gene interaction, which is affected by genotype. For example, excess riboflavin aggravates the level of DNA damage when folate is deficient and such effects are modulated by the common C677T polymorphism in the MTHFR gene.[23,24] These observations have led to the emerging science of genome health nutrigenomics, which is based on the principle that DNA damage is a fundamental cause of disease that can be diagnosed and nutritionally prevented on an individual, genetic subgroup, or population basis. Given the fundamental importance of genome integrity maintenance, it was proposed that dietary reference values should also be defined by the impact of nutritional deficiency or excess on genome integrity at the chromosomal, telomere, mitochondrial and DNA base sequence level.[21,22] Chromosomal aberrations can be measured in lymphocytes, and in fact this was the method used to show for the first time that protein calorie malnutrition increases chromosomal damage 5.5-fold in children.[25] Another method that is commonly used is measurement of micronuclei which arise from chromosome fragmentation or chromosome malsegregation during mitosis, and this method was shown to be sensitive to a wide range of deficiencies (e.g., folate, vitamin B_{12}, zinc deficiency) and also increased in obesity.[26,27,28] DNA strand breaks measured by comet or γH2AX assays and oxidation of guanine are also sensitive tools to assess genome damage effects of malnutrition.[29,30,31] More and more studies are also measuring effects on telomere length showing associations with nutritional deficiencies such as those of zinc, folate, vitamin D and omega-3 fatty acids.[32–35] Of these biomarkers, only telomere and micronucleus assays have been adequately validated, at this point in time, with respect to associations with nutritional status (both cross-sectionally and by controlled intervention) and with developmental and degenerative diseases (both via case-control and prospective cohort studies).[22]

Other "omic" biomarkers of nutritional status

Apart from DNA damage biomarkers, it is possible to utilize transcriptomic, proteomic and metabolic biomarkers to assess the impact of nutritional deficiency, excess or intervention. For example, a network of expressed genes associated with environmental exposures, nutritional intake and ageing can be successfully used to identify biomarkers that relate to the interactive effects of nutrition with life-stage and other environmental factors and to test the plausibility of a connection between nutritional factors and observed genomic changes.[36,37,38] For instance, nutritional deficiencies that increase oxidative stress might be linked with changes in expression of genes susceptible to guanine oxi-

FIGURE 3: The various "omic" biomarkers that are currently used to investigate the impact of nutrients and diets on human health at the cellular and organism level. This figure is an adaptation of that reported by DeFlora and Izzotti.[44,45]



dation in their promoter sequences.[7,8] Furthermore, it is becoming increasingly evident that cellular and metabolic responses to environmental changes including nutritional deficiency or excess are also mediated by micro RNA (miRNA) and that the miRNA "ome" may provide specific fingerprints of nutrient exposure. For example, it has been shown that the miRNAs miR-222, miR-10a and miR-let7b are up-regulated when folate, retinoic acid and vitamin D are deficient, respectively.[39] In a similar vein, plasma proteome analysis is yielding protein markers which are associated with specific deficiencies. For instance, the plasma proteins, RBP4, VDBP, RGS8, Cp and SEPP1 are associated with deficiencies in retinol, vitamin D, α-tocopherol, copper and selenium respectively.[40,41] Together with established metabolic biomarkers of nutritional deficiencies, it has now become possible to identify a network of biomarkers across all of the "omes" that can better diagnose the specific deficiencies, their interactions and likely pathologies at the genome level.[42,43]

Conclusions

The science of nutrigenomics and nutrigenetics is still in its infancy but its potential to improve our understanding of how to diagnose and treat nutritional deficiency or imbalance is already evident. More effort is required to utilize this rich knowledge appropriately to **(1)** better inform, validate and test the design of dietary pattern and fortification recommendations and **(2)** properly measure and rectify poor nutrition in both developing and developed countries, taking into consideration the genetic background of communities and the food supplies that are available to them. The various "omic" biomarkers that are now available need to become more accessible for epidemiological/intervention studies and, furthermore, they should be standardized and validated to improve their translation into public health practice.

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Correspondence: ***Professor Michael Fenech,***
Director, Personalised Nutrition and Genome Health Laboratory. CSIRO Food and Nutrition, PO Box 10041, Adelaide BC, SA 5000, Australia. ***Email:*** *michael.fenech@csiro.au*

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