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## Symposium on ‘Diet and cancer’

# Genome-health nutrigenomics and nutrigenetics: nutritional requirements or ‘nutriomes’ for chromosomal stability and telomere maintenance at the individual level

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It is becoming increasingly evident that (a) risk for developmental and degenerative disease increases with more DNA damage, which in turn is dependent on nutritional status, and (b) the optimal concentration of micronutrients for prevention of genome damage is also dependent on genetic polymorphisms that alter the function of genes involved directly or indirectly in the uptake and metabolism of micronutrients required for DNA repair and DNA replication. The development of dietary patterns, functional foods and supplements that are designed to improve genome-health maintenance in individuals with specific genetic backgrounds may provide an important contribution to an optimum health strategy based on the diagnosis and individualised nutritional prevention of genome damage, i.e. genome health clinics. The present review summarises some of the recent knowledge relating to micronutrients that are associated with chromosomal stability and provides some initial insights into the likely nutritional factors that may be expected to have an impact on the maintenance of telomeres. It is evident that developing effective strategies for defining nutrient doses and combinations or ‘nutriomes’ for genome-health maintenance at the individual level is essential for further progress in this research field.

### DNA damage: Folate: Nicotinic acid: Poly(ADP-ribose) polymerase

The central role of the genetic code in determining genome stability and related health outcomes such as developmental defects and degenerative diseases including cancer is well established<sup>(1–10)</sup>. In addition, it is evident that DNA metabolism and repair is dependent on a wide variety of dietary factors that act as cofactors or substrates in these fundamental metabolic pathways<sup>(1–5)</sup>. DNA is continuously under threat of major mutations from conception onwards by a variety of mechanisms that include: point mutation; base modification as a result of reactive molecules such as the hydroxyl radical; chromosome breakage and rearrangement; chromosome loss or gain; gene silencing as a result of inappropriate methylation of CpG at promoter sequences; activation of parasitic DNA expression as a result of reduced methylation of CpG; silencing of house-keeping genes as a result of DNA hypermethylation of

CpG islands in gene promoter regions; accelerated telomere shortening or dysfunction<sup>(6–8)</sup>. The main challenge to a healthy and long life is the ability to continue to replace senescent cells in the body with fresh new cells with normal genotypes and gene expression patterns that are tissue-appropriate. Understanding the nutritional requirements for genome-health maintenance of stem cells is essential in this context, but has so far not been adequately explored.

While much has been learnt of the genes involved in DNA metabolism and repair and their role in a variety of pathologies, such as defects in *BRCA1* and *BRCA2* genes that cause increased risk for breast cancer<sup>(9,10)</sup>, much less is known of the impact of cofactor and/or micronutrient deficiency or excess on the fidelity of DNA replication and efficiency of DNA repair. For example, a deficiency in a

**Abbreviations:** MTHFR, methylenetetrahydrofolate reductases; PARP, poly(ADP-ribose) polymerase; TANK, tankyrase; TL, telomere length; TRF, telomere repeat-binding factors.

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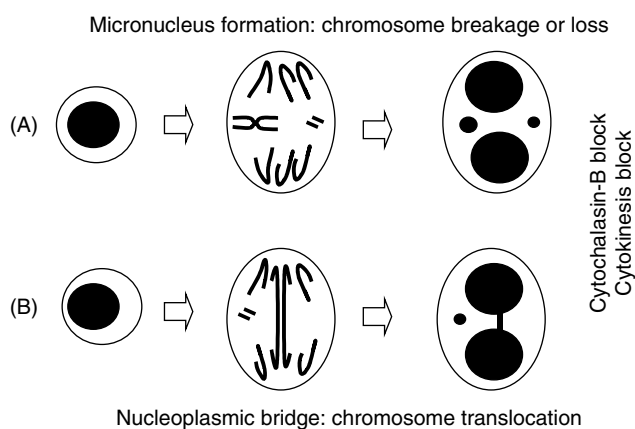
micronutrient required as a cofactor or as an integral part of the structure of a DNA repair gene (e.g. Zn as a component of the DNA repair glycosylase OGG1 involved in the removal of oxidised guanine or Mg as a cofactor for several DNA polymerases) could mimic the effect of a genetic polymorphism that reduces the activity of that enzyme<sup>(1–3)</sup>. Thus, nutrition has a critical role in DNA metabolism and repair, and this awareness is leading to the development of the new fields of genome-health nutrigenomics and genome-health nutrigenetics<sup>(5)</sup>. The critical aim of these fields is to define optimal dietary intakes for the prevention of DNA damage and aberrant gene expression for genetic subgroups and ultimately for each individual.

### Evidence linking genome damage with adverse health outcomes

Genome damage impacts on all stages of life. There is good evidence to show that infertile couples exhibit a higher rate of genome damage than fertile couples<sup>(11)</sup> when their chromosomal stability is measured in lymphocytes using the cytokinesis block micronucleus assay<sup>(12,13)</sup> (see Fig. 1). Infertility may be a result of a reduced production of germ cells because genome damage effectively causes programmed cell death or apoptosis, which is one of the mechanisms by which grossly mutated cells are normally eliminated<sup>(14–16)</sup>. When the latter mechanism fails reproductive cells with genomic abnormalities may survive, leading to serious developmental defects<sup>(17,18)</sup>. That an elevated rate of chromosomal damage is a cause of cancer has been demonstrated by ongoing prospective cohort studies in European countries that have shown a 2–3-fold increased risk of cancer in those individuals whose chromosomal damage rate in lymphocytes is in the highest tertile when measured 10–20 years before cancer incidence is measured<sup>(19)</sup>. It has also been shown that an elevated micronucleus frequency, a robust biomarker of chromosome breakage or loss, in lymphocytes predicts cancer risk in man<sup>(20)</sup>. Chromosomal damage is also associated with accelerated ageing and neurodegenerative diseases<sup>(10,21–27)</sup>. Those individuals with accelerated ageing syndromes as a result of redox imbalances (e.g. Down syndrome) and/or suboptimal DNA repair (e.g. carriers of deleterious mutations in the *ATM* or *BRCA1* genes) may be particularly susceptible to the genome-damaging effects of suboptimal micronutrient intake<sup>(28–31)</sup>.

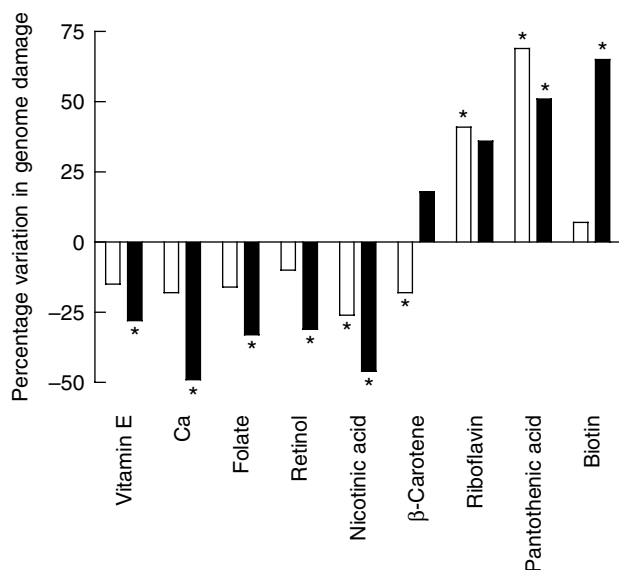
### The concept of genome damage as a marker of nutritional deficiency

There is overwhelming evidence that several micronutrients (vitamins and minerals) are required as cofactors for enzymes or as part of the structure of proteins (metaloenzymes) involved in DNA synthesis and repair, prevention of oxidative damage to DNA and maintenance methylation of DNA. The role of micronutrients in the maintenance of genome stability has recently been extensively reviewed<sup>(1,2,4,32)</sup>. The main point is that genome damage caused by moderate micronutrient deficiency is of



**Fig. 1.** Expression of micronuclei and nucleoplasmic bridges during nuclear division. Micronuclei originate from either (1) lagging whole chromosomes (A) that are unable to engage with the mitotic spindle because of a defect in the spindle or a defect in the centromere–kinetochore complex required to engage with the spindle, or (2) an acentric chromosome fragment originating from a chromosome break (A and B) that lags behind at anaphase because it lacks a centromere–kinetochore complex. Mis-repair of two chromosome breaks may lead to an asymmetrical chromosome rearrangement producing a dicentric (i.e. two centromeres) chromosome and an acentric fragment (B); frequently the centromeres of the dicentric chromosome are pulled to opposite poles of the cell at anaphase resulting in the formation of a nucleoplasmic bridge between the daughter nuclei. Nucleoplasmic bridges are frequently accompanied by a micronucleus originating from the associated acentric chromosome fragment. Nucleoplasmic bridges may also originate from dicentric chromosomes caused by telomere end fusions. As micronuclei and nucleoplasmic bridges are only expressed in cells that have completed nuclear division it is necessary to score these genome instability biomarkers specifically in once-divided cells. This process is readily accomplished by blocking cytokinesis using cytochalasin-B (for a more detailed explanation, see Fenech<sup>(7,12,13)</sup>).

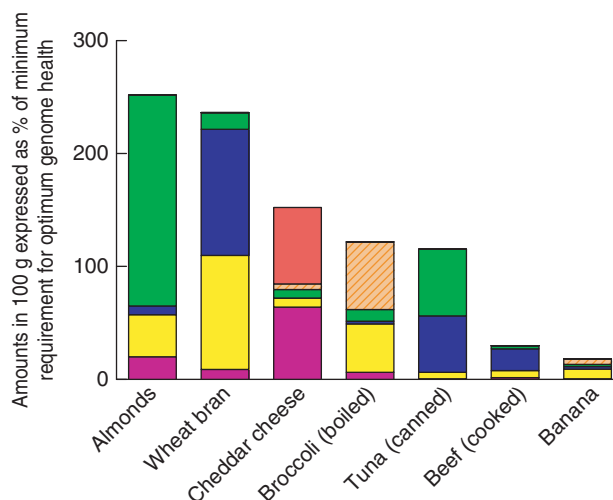
the same order of magnitude as the genome-damage levels caused by exposure to sizeable doses of environmental genotoxins such as chemical carcinogens, UV radiation and ionising radiation, about which there is already a heightened level of concern. A telling example from the authors' laboratory is the observation that chromosomal damage in cultured human lymphocytes caused by reducing folate concentration (within the normal physiological range) from 120 nmol/l to 12 nmol/l is equivalent to that induced by an acute exposure to low linear-energy-transfer ionising radiation (e.g. X-rays) at 0.2 Gy, a dose of radiation that is approximately ten times greater than the annual allowed safety limit of exposure for radiation workers<sup>(5)</sup>. If moderate deficiency in just one micronutrient can cause sizeable DNA damage it is reasonable to be concerned about the possibility of additive or synergistic effects of multiple moderate deficiencies on genome stability. Clearly, there is a need to start exploring the genotoxic effects of multiple micronutrient deficiencies, as well as excesses, which are prevalent in human populations. This aspect is analogous to genetic studies that explore, for example, the combined effects of polymorphisms in DNA repair genes on DNA damage.



**Fig. 2.** Percentage variation in genome damage for the mid-tertile of intake (□) and the highest tertile of intake (■) of vitamin E, calcium, folate, retinol, nicotinic acid, β-carotene, riboflavin, pantothenic acid and biotin relative to the lowest tertile of intake in an Australian cohort of healthy adults. Genome damage rate was measured in peripheral blood lymphocytes using the cytokinesis-block micronucleus assay (for more details, see Fenech *et al.*<sup>(33)</sup>). The percentage variations in genome damage were significant: \* $P < 0.006$ .

### Results from a recent population study suggest that at least nine micronutrients affect genome stability in human subjects *in vivo*

The results have recently been reported of a cytogenetic epidemiological study of 190 healthy individuals (mean age 47.8 years, 46% males) designed to determine the association between dietary intake, measured using an FFQ, and genome damage in lymphocytes<sup>(33)</sup>, measured using the cytokinesis-block micronucleus assay (see Fig. 2). Multivariate analysis of baseline data shows that (a) the highest tertile of intake of vitamin E, retinol, folate, nicotinic acid (preformed) and Ca is associated with significant reductions in micronucleus frequency (%), i.e. -28, -31, -33, -46 and -49 respectively (all  $P < 0.005$ ) relative to the lowest tertile of intake and (b) the highest tertile of intake of riboflavin, pantothenic acid and biotin is associated with significant increases in micronucleus frequency (%), i.e. +36 ( $P = 0.054$ ), +51 ( $P = 0.021$ ) and +65 ( $P = 0.001$ ) respectively relative to the lowest tertile of intake (Fig. 2). The mid-tertile of β-carotene intake is associated with an 18% reduction in micronucleus frequency ( $P = 0.038$ ); however, the highest tertile of intake (>6400 μg/d) results in an 18% increment in micronucleus frequency. There was an interest in investigating the combined effects of Ca or riboflavin with folate consumption because epidemiological evidence suggests that these dietary factors tend to interact in modifying the risk of cancer<sup>(34–36)</sup> and they are also associated with reduced risk of osteoporosis and hip fracture<sup>(37–39)</sup>. Interactive additive effects were observed, such as the



**Fig. 3.** Content of micronutrients associated with reduced DNA damage in selected common foods. The height of each bar for each micronutrient within the separate foods corresponds to the amount of the micronutrient expressed as the percentage of the minimum daily intake associated with a reduced micronucleus frequency index in lymphocytes as determined in the study of Fenech *et al.*<sup>(33)</sup>. The relative contribution of each of the micronutrients (if present) is indicated by the height of each specifically coloured bar. The nutrient content of the foods was determined using published food content tables<sup>(122)</sup>. (■), Calcium; (■), folate; (■), niacin; (■), vitamin E; (■), β-carotene; (■), retinol.

protective effect of increased Ca intake (-46%) and the exacerbating effect of riboflavin (+42%) on increased genome damage caused by low folate intake. The results from this study illustrate the strong impact of a wide variety of micronutrients and their interactions on genome health depending on the level of intake.

The amount of micronutrients that appear to be protective against genome damage vary greatly between foods (Fig. 3) and careful choice is needed to design dietary patterns optimised for genome-health maintenance. As dietary choices vary between individuals, because of taste preferences that may be genetically determined or cultural or religious constraints, several options are required and supplements may be needed to cover gaps in micronutrient requirements. Clearly, the development of nutrient-dense foods and ingredients, such as aleurone flour, which is rich in bioavailable folate as well as other micronutrients required for DNA replication and repair<sup>(40,41)</sup>, is essential in making it feasible for individuals to achieve their daily nutrient requirements for genome-health maintenance without intake of excess energy.

An important consequence of these considerations is also the need to start defining RDA for all nutrients based on prevention or minimisation of genome damage.

### Genome-health nutrigenomics and genome-health nutrigenetics

Two of the important emerging areas of nutrition science are the fields of nutrigenomics and nutrigenetics. The term nutrigenomics refers to the effect of diet on DNA stability

## Telomeres

and gene expression. The term nutrigenetics refers to the impact of genetic differences between individuals in their response to a specific dietary pattern, functional food or supplement for a specific health outcome. The specific fields of genome-health nutrigenomics<sup>(5)</sup> and genome-health nutrigenetics are proposed on the premise that a more useful approach to the prevention of diseases caused by genome damage is to take into consideration that (a) inappropriate nutrient supply can cause sizeable levels of genome mutation and alter expression of genes required for genome maintenance and (b) common genetic polymorphisms may alter the activity of genes that affect the bioavailability of micronutrients and/or the affinity for micronutrient cofactors in key enzymes involved in DNA metabolism or repair. Supplementation of the diet with appropriate minerals and vitamins could, in some cases, help overcome inherited metabolic blocks in key DNA maintenance pathways<sup>(42,43)</sup>. Increasing the concentration of a cofactor by supplementation is expected to be particularly effective when a mutation (polymorphism) in a gene decreases the binding affinity for its cofactor resulting in a lower reaction rate. The interaction between genotype and diet in modulating risk is emerging as an exciting area of research in relation to micronutrient effects on DNA. This position is illustrated by results from studies on the common mutations in the methylenetetrahydrofolate reductase (MTHFR) gene and other genes in the folate-methionine cycle in relation to their modulating affect on risk of developmental defects and cancer<sup>(44–46)</sup>. Recent results from the authors' laboratory have shown that there are important significant interactions between the MTHFR C677T polymorphism, its cofactor riboflavin and folic acid in relation to chromosomal instability<sup>(47)</sup>. This relationship is demonstrated by (a) the reduction in nuclear bud frequency (a biomarker of gene amplification) in TT homozygotes relative to CC homozygotes for the MTHFR C677T mutation and (b) the observation that a high riboflavin concentration increases nuclear bud frequency under low folic acid conditions (12 nm-folic acid), probably by increasing MTHFR activity, which diverts folate away from dTTP synthesis, increasing the odds for uracil incorporation into DNA, chromosome breaks, the generation of breakage–fusion–bridge cycles and subsequent gene amplification and nuclear bud formation<sup>(47)</sup>. Clearly, the relative impact of genetic factors and nutrients on genome maintenance and their interactions needs better understanding so that appropriate knowledge on the most critical factors is developed. *In vitro* studies on the interactive effects of folic acid deficiency and inherited mutations in the *MTHFR*, *BRCA1* and *BRCA2* genes indicate that moderate deficiency in folic acid has a stronger impact on genome instability, measured by the cytokinesis-block micronucleus assay, than these important inherited mutations<sup>(47,48)</sup>, which again emphasises the magnitude of the impact of diet on genome maintenance. While cytogenetic assays such as the cytokinesis-block micronucleus assay are the most practical tools to study the effects of nutrients on chromosomal instability, further insights into the effects of nutrients on the genome may be obtained by studying alterations to critical regions of chromosomes such as the telomeres.

Telomeres are nucleoprotein structures that cap the ends of chromosomes, maintain chromosome stability and prevent end-to-end fusion of chromosomes during cell division<sup>(49–52)</sup>. These structures play a pivotal role in maintaining overall chromosome stability, as well as triggering a signal for normal ageing cells to senesce when telomeres become dysfunctional<sup>(52)</sup>. Degradation of telomeres has been shown to lead to whole chromosomal instability, via telomere end fusion, and the generation of breakage–fusion–bridge cycles within chromosomes, which leads to gene amplification and gene dosage imbalance, an important risk factor for cancer<sup>(53–55)</sup>. Telomere shortening has also been proposed as one of the fundamental mechanisms that determine the rate of ageing in cells<sup>(50,56,57)</sup>. Extensive evidence demonstrates the impact of dietary and environmental factors on chromosome stability<sup>(32,33,58–63)</sup>; however, there is limited knowledge of their impact specifically on telomere length (TL) and telomere structural integrity. Accordingly, knowledge of the impact that dietary and environmental factors have on telomeres is important for the maintenance of stem cells in a genomically-stable condition, as well as being crucial for the prevention of degenerative diseases of ageing, immune dysfunctions and cancers. The present section and subsequent sections of the present review will outline the structure of the telosome (the telomere and associated proteins) and discuss the potential impact of dietary deficiencies on this structure. In particular, there will be discussion of the plausibility that folate and nicotinic acid deficiencies together with increased oxidative stress may accelerate telomere dysfunction. Such metabolic imbalances may possibly explain the observed associations between telomere shortening and a number of conditions including obesity, psychological stress, immune dysfunction, cancer and CVD<sup>(64–69)</sup>.

### Telomere structure and the telosome

Telomeric DNA in vertebrates is composed of tandem repeats of the hexamer (TTAGGG)<sub>n</sub>, 8–15 kb in length in human subjects<sup>(52)</sup>. As a result of the end-replication problem, whereby DNA polymerases are unable to copy the final linear stretch of the lagging strand, each time DNA is replicated a small stretch of telomeric DNA is lost<sup>(70)</sup>. When TL is critically short a DNA damage response is triggered that results in chromosomal end-to-end fusions or cell arrest and apoptosis<sup>(50,52)</sup>. The rate of telomere shortening appears to vary between age-groups, between genders and even between chromosomes of the same cells, with each chromosome arm having an age-specific TL and erosion pattern<sup>(71)</sup>. This variation results in a heterogeneity in chromosome-specific TL shortening with age. On average, males have shorter telomeres with age and a faster rate of telomere loss compared with females in all age-categories, a possible factor influencing the differences in life expectancy between the genders<sup>(71)</sup>.

The structure of telomeres involves a T-loop at the end of the chromosome, formed by the 3'OH G-strand overhang<sup>(52,72,73)</sup>. This structure protects the telomere from

degradation by nucleases and minimises the possibility of it being mistakenly targeted as a double strand break requiring repair<sup>(74)</sup>. A number of proteins are associated with telomeric DNA. These proteins are collectively known as the shelterin complex or the telosome. The key proteins include telomere repeat-binding factors (TRF) 1 and 2, TRF1-interacting nuclear factor, repressor/activator protein and protection of telomeres 1<sup>(75)</sup>. TRF1 and TRF2 both bind directly to double-stranded DNA and form complexes at either end of the telomere<sup>(52,75)</sup>. TRF1 forms a multiprotein complex incorporating tankyrase (TANK) 1 and other poly(ADP-ribose) polymerase (PARP) molecules that play a role in TL maintenance<sup>(52)</sup>. The TRF2 complex binds to a long (150–200 nucleotides) 3'OH G-strand overhang and plays a role in protecting the chromosome from end fusion to other chromosomes<sup>(52,76)</sup>, with TRF1-interacting nuclear factor providing a bridging structure between TRF1 and TRF2<sup>(77)</sup>.

### Telomere length, telosome integrity and disease

Shortened telomeres result in a high level of chromosome instability, leading to loss of cell viability, and are associated with ageing-related pathologies including heart failure, immunosenescence (infections), digestive tract atrophies, infertility, reduced viability of stem cells, reduced angiogenic potential, reduced wound healing and loss of body mass<sup>(52,70)</sup>. Several premature-ageing syndromes, such as Werner and Bloom syndrome and aplastic anaemia, show an accelerated rate of telomere shortening, resulting in an early onset of ageing-related pathologies<sup>(52)</sup>. Shortened telomeres have been strongly implicated in breast, prostate and colo-rectal cancers and certain leukaemias as a consequence of mitotic disturbances such as structural rearrangements of chromosomes, loss of whole chromosomes, and nucleoplasmic bridges arising from end fusion<sup>(78–85)</sup>.

Telomerase, a reverse transcriptase, extends telomeres, replacing lost telomeric DNA. It is usually only substantially expressed in certain cells in the body, such as stem cells and germ cells, where it is necessary to maintain TL at approximately 15 kb<sup>(86)</sup>. The enzyme consists of two main subunits, telomerase reverse transcriptase and telomerase RNA component. Telomerase recognises and binds to the 3'OH overhang, where it then extends the DNA using an RNA molecule as a template, encoded by the telomerase RNA component subunit<sup>(52)</sup>. Late-stage cancer cells often have very short telomeres, indicative of their long proliferative history; however, 80–90% of these cells have been shown to have active telomerase, thus facilitating their immortality<sup>(52,87)</sup>. As a result, blocking telomerase activity is of great interest as a cancer therapy, as it may be targeted without affecting healthy somatic cells that do not normally express telomerase to the same extent as cancer cells<sup>(88)</sup>. An alternative mechanism for telomere elongation, termed ALT, exists whereby DNA from one telomere anneals with the complementary strand of another, acting as a primer for the synthesis of new telomere repeat sequences<sup>(87)</sup>. This mechanism has been shown to be active in some telomerase-negative cancer

cells, and may in some circumstances function in addition to active telomerase<sup>(87)</sup>.

The addition of new repeat sequences at telomeres is a tightly regulated process for maintaining TL within a certain range, as well as ensuring senescence signals occur at an appropriate point in the life of the cell. Deletion mutant studies conducted in *Saccharomyces cerevisiae* indicate that >150 genes may be involved (directly or indirectly) in the regulation of TL, with approximately one-third of these genes resulting in telomere elongation<sup>(89)</sup>. Substantial alterations in the expression or binding capacity of telosome proteins may modify access to telomerase and the extent of telomere elongation<sup>(77,90)</sup>. Epigenetic controls are another mechanism that has been shown to play an important role in telomere integrity, with mouse models deficient in DNA methyltransferases showing dramatically elongated telomeres compared with wild-type controls<sup>(91)</sup>. The lack of DNA methyltransferases results in an increase in telomeric recombination (indicated by sister-chromatid exchanges involving telomeric sequences) and the presence of ALT-associated promyelocytic leukaemia bodies, an event common to specific cancers<sup>(91)</sup>. Telomeric DNA repeats are typically unmethylated at cytosine, while subtelomeric DNA is usually heavily methylated. Reduced DNA methyltransferase activity results in hypomethylation of subtelomeric repeats, altered configuration of the proteins associated with the telomere, increased homologous recombination of telomeric sequences, increased access of telomerase to the telomeric DNA and an increase in TL. These observations suggest that methylation of subtelomeric DNA may play a role in maintaining telomere structural stability by repressing homologous recombination and possibly prevention of excessive telomere elongation and telomere end fusions via recombination<sup>(91,92)</sup>.

### Folate and telomere integrity

Folate is essential in the cell for the synthesis of dTTP from dUMP<sup>(4,93)</sup>. Under folate-deficient conditions uracil is incorporated into DNA instead of thymidine, leading to chromosome breakage and micronucleus formation<sup>(93–96)</sup>. Glycosylases excise uracil from the newly-synthesised strand resulting in abasic sites and DNA damage because simultaneous excision of uracil and/or other damaged DNA bases on opposite strands of DNA within twelve bases of each other has been shown to result in double-strand DNA breaks<sup>(32,93–95)</sup>. Several *in vitro* studies have demonstrated that human cells show an inverse dose-dependent correlation between concentration of folate in the physiological range and increased DNA damage such as micronuclei (biomarker of double strand breaks) and nucleoplasmic bridges (biomarker of chromosome fusions and/or breakage–fusion–bridge cycles)<sup>(94,96)</sup>. Nucleoplasmic bridges are expected to originate from dicentric chromosomes caused by telomere end fusions and/or misrepair of DNA strand breaks and highlight the possibility that folate deficiency may, in some way, cause instability at the telomeric ends of chromosomes. Recent studies in yeast suggest that insufficient synthesis of dTTP from dUMP could result in shortened TL<sup>(97)</sup>, raising the

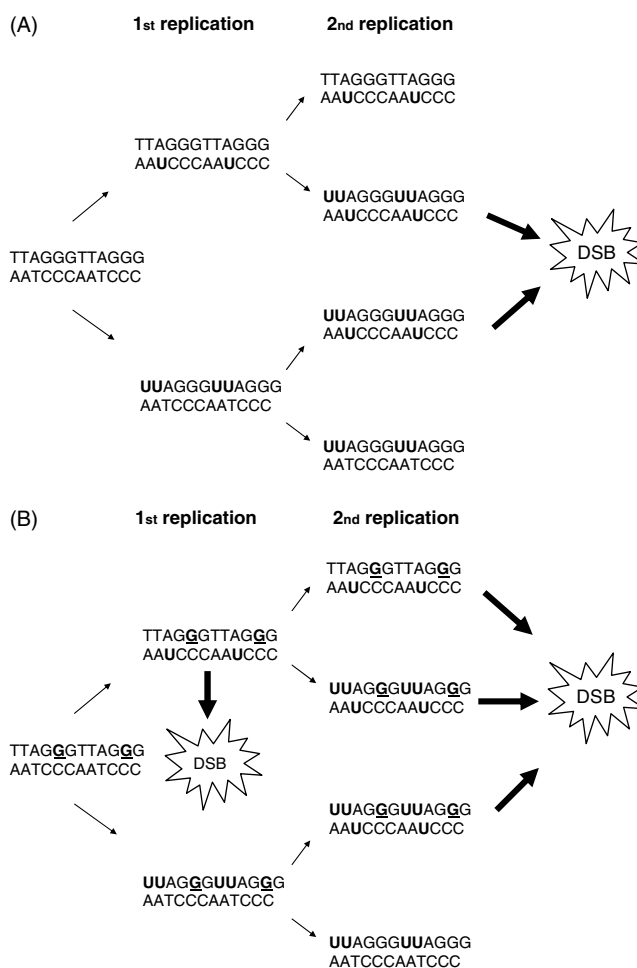
possibility that excessive uracil incorporation might cause breaks in the telomere sequence, as it does in other regions of the chromosome. Similarly, oxidative stress in mammalian and human cells has also been shown to cause telomere shortening, possibly as a result of the creation of abasic sites via glycosylases (discussed in following Section)<sup>(98,99)</sup>. Consequently, under low folate conditions the thymidine-rich telomere repeat sequence may be particularly prone to DNA breaks, leading to telomere shortening thus increasing chromosomal instability and DNA damage (Fig. 4).

Folate and other methyl donors such as vitamin B<sub>12</sub>, choline and methionine also play a critical role in maintenance methylation of cytosine which, apart from its importance for transcriptional control of gene expression, determines the structural stability of important regions of the chromosomes such as the centromeres and the sub-telomeric DNA. As discussed earlier, there is strong evidence that defects in the DNA methylation process can cause excessive telomere elongation and homologous recombination between telomeres that could lead to telomere end fusions<sup>(91)</sup>. It is therefore possible that deficiency of folate and other methyl donors may also result in telomere instability by causing inadequate maintenance of methylation in the sub-telomeric sequences, which leads to telomere dysfunction. It is also plausible that hypomethylation or hypermethylation of the CpG islands in the promoter of telomerase may cause excessive expression of telomerase or silence the gene respectively in differentiated cells<sup>(100)</sup>.

#### Nicotinic acid and telomere integrity

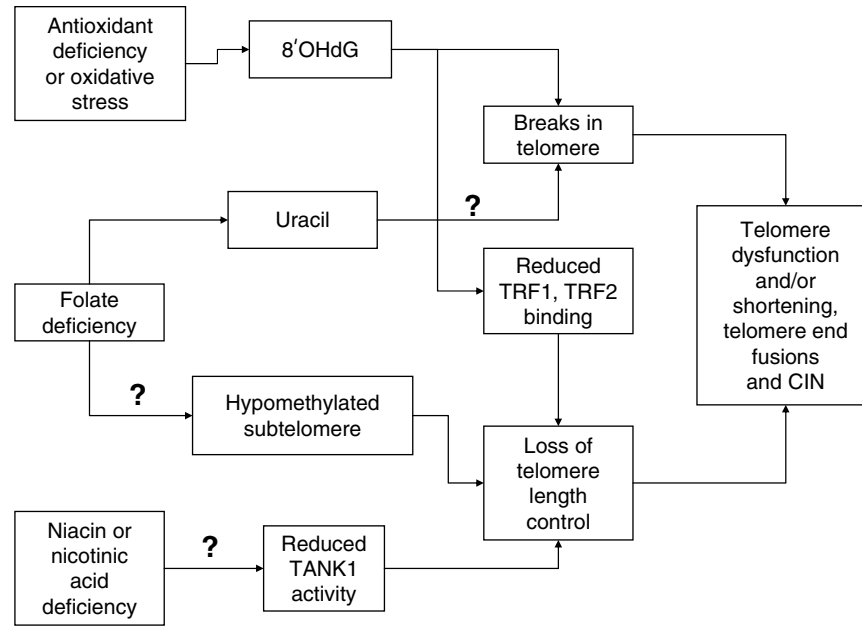
Nicotinic acid (niacin) is another dietary micronutrient that is known to play a fundamental role in chromosome integrity and reduction of cancer risk<sup>(101,102)</sup>. PARP is a DNA break sensor, a deficiency of which has been shown to cause telomere shortening and chromosome instability<sup>(103–106)</sup>. Activation of the PARP molecule occurs upon binding of its two Zn finger domains to single or double DNA strand breaks<sup>(104)</sup>. Activation results in automodification by attachment of ADP-ribose moieties sourced from NAD<sup>(101,104)</sup>. Long polymers,  $\leq 200$  units and often branching, are added to the PARP molecule as well as to other DNA repair proteins that are recruited to the complex. The complex is dynamic, being rapidly broken down by poly-ADP glycohydrolase, thus freeing up ADP-ribose units. The process of poly(ADP-ribosylation) requires nicotinic acid as a precursor of NAD, which is consumed during this process giving rise to nicotinamide<sup>(104)</sup>.

At least seven members have been identified in the PARP family<sup>(104)</sup>, three of which have been shown to associate with telomeric protein complexes at various stages of the cell cycle (PARP1, PARP2 and TANK1)<sup>(107–110)</sup>. TANK1 is a human telomere-specific PARP that is present at multiple subcellular sites including telomeres, mitotic centrosomes, nuclear pore complexes and the Golgi apparatus<sup>(109)</sup>. TANK1 positively regulates TL through its interaction with TRF1<sup>(111,112)</sup>. TRF1-interacting nuclear factor exercises a higher level of control by inhibiting



**Fig. 4.** Possible models of strand breaks in telomere DNA sequence caused by base excision repair of damaged bases such as uracil (**U**) and oxidised guanine (**G**). (A) Folate deficiency causes a high dUMP:dTMP in the cell, resulting in increased **U** incorporation into DNA instead of thymidine. **U** bases are then excised by uracil glycosylase, leading to abasic sites and double-strand breaks (DSB) in DNA during the base excision repair process if **U** is present on complementary DNA strands within twelve bases of each other<sup>(93,95)</sup>. In the model shown, this situation may occur after two cell divisions under folate deficiency conditions. (B) Combined effects of oxidative stress and folate deficiency. Oxidative stress causes oxidation of DNA bases such as 8'-hydroxydeoxyguanosine (8'-OHdG). Oxidised bases, such as **G**, are excised by glycosylases, resulting in the formation of an abasic site and DSB in DNA during base excision repair. Under low folate conditions this process may result in a DSB within one cell division cycle if the DNA incorporates **U** when it already contains oxidised bases<sup>(98,99)</sup>.  $\blackrightarrow$ , The formation of DSB within the telomere sequence if base excision repair occurs to remove **U** and **G** simultaneously on the opposite strands of the telomeric DNA.

the previously mentioned interaction<sup>(111)</sup>. Binding of TANK1 to TRF1 occurs via one or more of five discrete binding sites, followed by poly(ADP-ribosylation) of TRF1 resulting in a negative charge, thereby inhibiting its binding to telomeric DNA<sup>(108)</sup>. Once released from DNA, TRF1 is ubiquitinated and degraded by proteasomes, directly reducing the level present in the cell<sup>(108,113)</sup>.



**Fig. 5.** Possible mechanisms by which deficiency of folate and/or niacin (or nicotinic acid) and/or antioxidants may cause dysfunction of telomeres and consequently chromosomal instability (CIN) as a result of telomere end fusions. 8'OHdG, 8'-hydroxydeoxyguanosine; TANK, tankyrase; TRF, telomere repeat binding factor; ?, plausible but untested mechanisms.

Overexpression of TANK1 in the nucleus of telomerase-positive cells promotes an increase in TL; however, no effect has been observed in telomerase-negative human cells. This finding suggests that the activity of TANK1 involves regulating access of telomerase to the telomeric complex<sup>(112)</sup>. The recent identification of TANK2, a closely-related homologue of TANK1, opens the possibility for a second PARP being involved in telomere maintenance<sup>(112)</sup>.

In addition to regulating TL, another role for TANK1 at telomeres has been identified using a mouse knock-down model in which the absence of TANK1 results in mitotic arrest<sup>(108)</sup>. It was found that sister chromatids successfully separate at the centromere and chromosomal regions, but are unable to separate at the telomeres, causing arrest at early anaphase<sup>(108)</sup>. A later study has shown that nuclear mitotic apparatus protein is a major acceptor of poly(ADP-ribosylation) by TANK1 and may play a role in the resolution of sister chromatids and the exit from mitosis<sup>(109)</sup>. A defective TANK1 may thus also result in the formation of telomere end fusions and nucleoplasmic bridges at anaphase and therefore cause major chromosomal instability.

Given the pivotal role that PARP molecules play in maintaining chromosome integrity by recruiting repair protein complexes, regulating TL and facilitating separation of sister chromatid telomeres during anaphase, the need for adequate nicotinic acid levels to maintain the poly(ADP-ribosylation) process is likely. One study has investigated the effect of nicotinic acid deficiency on chromosomal instability in rats by using the micronucleus assay. Animals were fed one of three diets: nicotinic acid deficient; nicotinic acid replete; nicotinic acid

supplemented in conjunction with the cancer drug etoposide. A 6-fold increase in chromosomal instability was found in the nicotinic acid-deficient group compared with the nicotinic acid-replete group<sup>(114)</sup>. Supplementation over and above the replete levels was not found to have an additional effect. Similarly, a recent cross-sectional study has shown that micronucleus frequency in human lymphocytes *in vivo* is directly and inversely correlated with daily intake levels of nicotinic acid<sup>(33)</sup>. However, a human study conducted in twenty-one healthy smokers supplemented with four different levels of nicotinic acid has shown no protective effect against DNA damage<sup>(115)</sup>. Studies have been conducted into the epigenetic effects of NAD restriction, mainly focusing on its role as a cofactor for sirtuins, histone deacetylase enzymes<sup>(116–118)</sup>. No studies have been conducted that have specifically addressed the issue of TL and chromosomal instability under nicotinic acid-deficient conditions. The plausibility that niacin or nicotinic acid deficiency may impact on TL needs to be tested.

### Oxidative stress and telomere integrity

Reactive oxygen species such as the highly-reactive hydroxyl radical and superoxide radical have been implicated in numerous disease states<sup>(119)</sup>. Damage to DNA, lipids and proteins induced by reactive oxygen species may be controlled by antioxidants (such as vitamins C and E) as well as enzymic mechanisms such as superoxide dismutase, catalase and glutathione peroxidase<sup>(119)</sup>. In the case of lipids and proteins normal turnover will usually remove most damaged molecules, whilst oxidised DNA bases must

be rapidly identified and repaired to avoid the formation of point mutations in DNA during replication, which could lead to pathological phenotypic changes if critical genes are affected. This process occurs by the coordinated recruitment of components of the base excision repair pathway, including glycosylase, endonuclease, polymerase and ligase proteins<sup>(119)</sup>. Under certain conditions, such as increased levels of free radicals and/or reduced levels of antioxidants, an imbalance can occur and the capacity for prevention and repair can become overwhelmed.

Both the bases and the ribose components of DNA have been identified as targets for different forms of oxidative damage; however, guanine residues have been shown to be particularly prone, with 8'-hydroxydeoxyguanosine being a common biomarker of oxidative stress<sup>(119)</sup>. Given the high incidence of guanine residues in telomeric DNA it can be speculated that telomeres may be particularly sensitive to oxidative damage. Telomere shortening has been observed in cell lines under hyperoxic stress conditions (40% O<sub>2</sub> partial pressure), caused by increased telomere attrition from 90 bp per population doubling to 500 bp per population doubling<sup>(120)</sup>. Importantly, this study has also shown that TL following hyperoxic treatment became as short as those of senescent cells (approximately 4 kb) and the mechanism for attrition has been shown to be an accumulation of single-strand breaks<sup>(120)</sup>. *In vivo* evidence has also been found to support the theory of an increased rate of telomere shortening in conditions of ill-health that are associated with increased oxidative stress<sup>(64–69)</sup>. In *in vitro* studies antioxidant treatment has been found to prevent telomere attrition and high expression of superoxide dismutase is also associated with decreased telomere erosion rates and increased cellular lifespan<sup>(121)</sup>; however, whether similar effects can be achieved *in vivo* remains uncertain.

In order to explore the mechanism of oxidative stress on telomere loss a recent study has tested the effects of site-specific 8'-hydroxydeoxyguanosine lesions and the presence of base excision repair intermediates (e.g. abasic sites) within telomeres<sup>(90)</sup>. Single 8'-hydroxydeoxyguanosine lesions were found to reduce the percentage of bound TRF1 and TRF2 by  $\geq 50\%$  compared with undamaged telomeric DNA. Even more dramatic effects were observed with multiple 8'-hydroxydeoxyguanosine lesions<sup>(90)</sup>. Both abasic sites, as well as modified guanine residues, were found to disrupt binding of TRF1 and TRF2 proteins. Alteration of any of the guanines in the TTAGGG sequence was found to decrease binding; however, alteration of the third guanine shows the strongest effect<sup>(90)</sup>. TRF1 and TRF2 proteins have been shown to bind directly to DNA with a high extent of specificity and are critical for the recruitment of other protein components of the telosome<sup>(75)</sup>. Accordingly, oxidative damage to the telomere sequence may be expected to induce disruption of telomere capping and cause loss of telomere function. Whether oxidative damage to other bases in the telomere sequence also disables TRF1 and TRF2 binding remains to be tested.

Based on these considerations there are several plausible mechanisms involving folate and/or niacin (nicotinic acid) deficiency and/or antioxidant deficiency that could lead to

telomere dysfunction, which are summarized together in Fig. 5.

## Conclusion

It is evident that micronutrients play an important role in genome maintenance. Given that damage to the genome is the most critical pathological event that can lead to aberrant phenotype and tissue dysfunction, it is essential that more attention is given to the nutritional requirements for genome-health maintenance. As there are now excellent diagnostics for DNA damage, it is feasible to define the optimal nutritional requirements or 'nutriome' for individuals using DNA damage biomarkers and to determine whether their dietary choices are beneficial or harmful to their DNA. There are currently no reported investigations that have specifically focused on nutrient deficiencies and telomeric integrity. Determining the optimal micronutrient requirements for telomere maintenance may help to define more precisely the dietary strategies for optimising the genome health status of both differentiated cells and stem cells. Defining optimal culture conditions for genome-health maintenance of stem cells before their use *in vivo* for therapy is also critically important in preventing the risk of transplanting cells that may be prone to become cancers because of genomic instability acquired *in vitro*. Given the evidence that has been provided here it is postulated that optimising nutritional and lifestyle requirements for the prevention of chromosomal and telomere aberrations on an individual basis via 'genome health clinics'<sup>(5)</sup> could be one of the most cost-effective strategies for the prevention of developmental and degenerative diseases from now and into the future.

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