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Reflections in Mutation Research

A lifetime passion for micronucleus cytome assays—Reflections from Down Under[☆]Michael Fenech^{*}

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ABSTRACT

A brief account of an improbable career in the field of genetic toxicology is given, extending from my early years in Malta through a life-changing decision to study in Australia (Down Under). I describe the circumstances that led to the discovery of the cytokinesis-block micronucleus (CBMN) assay and its evolution into a cytome assay of chromosome breakage and loss (micronuclei), asymmetrical chromosome rearrangements or telomere end fusions (nucleoplasmic bridges), gene amplification (nuclear buds), cell death (necrosis, apoptosis) and cytostasis (nuclear division index). This paper also describes the role of my laboratory in the beginning of the HUMN project, its achievements, and the applications of CBMN cytome assays in the fields of radiation biology, genetic toxicology, epidemiology, biodosimetry and genome health nutrigenomics, leading to the Genome Health Clinic concept. Along the way I mention my encounters with some of the influential people in the field of mutagenesis who provided me with the motivation and guidance needed to realise these achievements. I hope this account provides some inspiration to the next generation of scientists who may be fortunate to see the realisation of the application of the principles of mutagenesis in health optimisation or disease prevention and eventually in mainstream medicine.

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1. The formative years

In my wildest dreams I would have never imagined that my life in research would turn out as it has. The first 25 years of my life were spent in Malta where I was born. That I had an inclination for biological sciences was evident in my very early years when I was fascinated by the response of ants to environmental stress and their ability to survive extreme conditions. There had never been any professional scientists in my family, but on my mother's side two uncles were doctors, and on my father's side one uncle was a pharmacist. My father was a health inspector, and my mother trained as a nurse. Therefore, by and large, I was exposed to issues underlying the importance of health maintenance, and growing up in the Mediterranean there was always strong awareness of the importance of diet, although the prevailing philosophy was the more you ate the better, which explains the high incidence of obesity and diabetes in Malta. Malta is a densely populated island

(316 km², population 402,000), and traffic fumes are a major pollution problem on busy roads such as the one I lived on in Birkirkara. Furthermore it was common when swimming to find your legs stained with tar residues from oil tankers that criss-crossed the Mediterranean sea. It was these experiences that first stimulated my curiosity about scientific issues relating to the effects of environmental pollution and diet on human health.

I graduated with a Bachelor's degree in Chemistry and Biology from the University of Malta and during this period developed an interest in such aspects of marine biology as measuring the impact of pollution on marine organisms and understanding the ecology of the rocky shores around the island of Malta. The latter was the focus of my Master's degree, in which I studied the zonation of organisms in the intertidal zone in the south of the island as well as the structure and function of the vermetid sessile mollusc *Serpulorbis arenaria* which was present in two polymorphic forms. This was my first great experience in basic research under the guidance of Dr. Victor Jaccarini who was in charge of the marine biological station located in one of the great fortifications, Fort St. Lucian, built by the Knights of the Order of St. John. My hope was to carry on my career in marine biology and ecology but these hopes were dashed when the government changed. The Socialist Party that took power believed that arts and science faculties at the university were a waste of money and decided amongst other

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things that the marine biological station should be closed down and replaced by a maritime museum, even although this meant losing a facility which was heavily used and held in very high regard by research groups internationally.

During the Master's degree period I was also teaching at pre-university level and developed an interest in genetics, and a short while later I took up a position as a scientist in the pathology laboratories at the island's main hospital, St. Luke's. Malta has a great tradition in medicine because of the influence of the Knights of the Order of St. John, which was set up initially as a hospitaller order to care for pilgrims travelling to Jerusalem and the Holy Land. The Knights transferred their headquarters from the island of Rhodes to Malta, which was offered to them after they were evicted from Rhodes in 1522 by the Ottomans under Suleiman the Magnificent. The Knights settled in Malta in the early 1500s and built massive fortifications as well as establishing one of the first medical universities in Europe. The experience at St. Luke's Hospital exposed me to the importance of haematology in health assessment, and it was here that for the first time I came face to face with the human lymphocyte and the Howell Jolly body (micronucleus) in erythrocytes. Little did I realise then that my life would become consumed by almost three decades of research devoted to the measurement of DNA damage in human lymphocytes using the micronucleus (MN) assay.

2. From Malta to Australia

At that time Malta offered relatively limited opportunity for individuals with active, unconventional and inquisitive scientific minds and it was not possible to pursue a Ph.D. on the island as the university had inadequate facilities for these purposes in science. Consequently, I started to explore possibilities for further studies overseas in the United Kingdom, but possibly also in other English-speaking countries. Scholarships were limited but I managed to secure one to study in Australia which was my least preferred choice because of distance from home, but it turned out to be an excellent experience and opportunity. With the limited information available to me I came to the conclusion that Adelaide was one of the better centres in Australia to do research in genetics, which had become my key area of interest. Through a series of coincidences, my letter of enquiry to the University of Adelaide ended up with Professor Avon Maxwell Clark at Flinders University who was interested in using the bone-marrow MN assay to study the genotoxic effects of alkaloids from drought-tolerant weeds toxic to sheep and cattle such as *Echium plantagineum* and *Heliotropium europeum*. Professor Clark was about to retire so he forwarded my enquiry to Alexander Morley who was then Professor of Haematology at the Flinders University Medical School and was soon to become internationally renowned for his research on the measurement of point mutations in human lymphocytes using the HPRT and HLA assays. Professor Morley (Alec) wrote back indicating that he would be happy to be my Ph.D. supervisor given my interest in genetics and experience in haematology. I could not believe my luck and reasoned that if I was to see the world I might as well start from one of the most distant places from Malta possible. New Zealand apart, this was Australia. My parents were somewhat devastated as I was the first member of the extended family to migrate, and naturally I was somewhat apprehensive given that I had no relatives "Down Under"—but I could not resist the adventure.

The journey to Adelaide in late April 1980 was memorable with a stop-over in Bangkok which at the time was on a high state-of-alert due to political unrest. It was my first experience of a tropical climate, and it was a complete contrast to the very dry and hot summers in Adelaide. It took me about 2 weeks to

recover from the long flight to Adelaide due to both the physical and emotional stress. The 1st week in Adelaide was unforgettable as I was unable to communicate properly because, despite the friendly reception and welcome by Alec and his team, my accent and body language when using English language were out of synchrony with the Australian way of speaking. Dr. David Turner, Kevin Trainor, Jack Dempsey, Alexander Kutlaca, Chris Matthews, Monica McCarron and Sheila Phillips eventually straightened me out, and I slowly settled into the research laboratory life which was a completely new experience to me. My attempts to break the ice in communication were eventually rewarded when I decided to use some long-forgotten Australian colloquialisms during our weekly research meeting such as "You must have kangaroos in your top paddock!" meaning "You must be crazy to have that thought in your head!". Participating in "happy hour" at the hospital common room during this early period on Friday afternoons was also a necessary and cheerful short-cut to get to know the younger and established research staff at Flinders Medical Centre where our research group was located.

3. The tortuous path to micronucleus assays

My initial 3–6 months in the Morley lab proved fruitless as my attempts to develop a novel point mutation assay based on the ABO blood group system did not meet with success. Nevertheless this project had required my temporary transfer to the donor–recipient blood cross-matching laboratory where I met my future wife Janet, and in this sense, my research failure was very much a success on the social and personal level. Professor Morley then suggested that perhaps I should change tack completely and pushed in front of me the paper of Countryman and Heddle [1] which had described for the first time the development of a MN assay in cultured lymphocytes. My failure with the ABO blood group point mutation system was to launch me into a future of great opportunity with the MN assay in lymphocytes.

I set about using Countryman and Heddle's conventional MN assay in lymphocytes and verified their results for ionising radiation. However, it soon became apparent that the results depended on harvest time, and false negative results could be generated under conditions of strong inhibition of mitosis as often occurs with chemical exposure. Furthermore it was evident that a greater proportion of lymphocytes respond to mitogen in cultures of lymphocytes from young subjects than in those from elderly individuals. In my second annual assessment seminar I hypothesised that the MN assay in its then-current form had a major flaw due to the fact that observed MN frequency depended on the proportion of lymphocytes that responded to mitogen as well as the number of divisions that occurred during the culture period prior to harvesting cells. I predicted that the assay would only be robust if a method to accumulate and identify once-divided cells was developed. My examiners recognised this and wisely suggested that if I solved this problem my Ph.D. would be secured. Naturally this predicament focuses the mind beautifully, and I set about devising a variety of methods to identify once-divided cells based on DNA synthesis labelling or parallel cultures blocked in mitosis to correct for the proportion of dividing cells. Neither of these methods was satisfactory because one could not be certain whether the labelled cells had actually completed nuclear division or had, in fact, completed only one division. Alec jokingly said, perhaps with the intention to spur me on, that "the conventional MN assay might be like a jacket that does not fit because whichever attempt to fix it generates another problem". Feeling somewhat optimistic that the solution would soon become evident, I responded that "It depends on how good the tailor is".

4. Eureka! The cytokinesis-block micronucleus (CBMN) assay

After considerable frustration, the “eureka” moment occurred to me very early one summer morning in 1984. It suddenly became obvious to me while half awake, almost in a dream, that the point at which to identify once-divided cells is the short-lived binucleated stage in telophase. That same day I enquired with colleagues whether anybody had any idea how to block and accumulate cells in this stage. I wondered how one could block cytokinesis so that the cell, having completed nuclear division, would no longer be able to complete cellular division. A quick search of the term “cytokinesis” in the Lehninger “Principles of Biochemistry” undergraduate textbook resulted in my finding a brief sentence mentioning cytochalasins as inhibitors of cytokinesis. They exerted this effect by inhibiting the polymerisation of actin into the microfilaments required for cytokinesis. The university library happened to have a textbook on cytochalasins that included detailed information on the various forms of these molecules produced by a wide range of fungi. It was evident from this information, as well as from a key paper by Carter [2], that cytochalasin-B was the form that could inhibit cytokinesis most efficiently in lymphocytes and other mammalian cells.

It happened that colleagues in the Immunology Department had some cytochalasin-B which they were using for enucleation experiments. That same day I added cytochalasin-B at 3 µg/ml to ongoing lymphocyte cultures, and the following morning I harvested the cells and prepared slides. I distinctly remember those first moments looking down the microscope and my excitement in seeing that a very large proportion of the lymphocytes were blocked as binucleated cells. I also recall showing the slide to Alec who also was pleasantly surprised by this development. Thus, the CBMN assay was born. Fig. 1 shows the concept of the CBMN assay and examples of binucleated cells containing micronuclei.

The following year was spent verifying the efficacy of the CBMN technique in terms of reproducibility and sensitivity for detecting low dose exposures to ionising radiation (5–40 cGy) and demonstrating that the rate of increase of MN frequency with age was underestimated in the conventional method relative to the CBMN method. I found that acute doses as low as 0.05 Gy X-rays were easily detectable by the new method which required much less effort than the more laborious metaphase analysis approach [3,4].

5. Postdoctoral research

After being awarded my Ph.D. I remained in Alec's laboratory for a further 2 years during which research performed in collaboration with Dr. Jim Denham at the Royal Adelaide Hospital led to the utility of the CBMN assay for biological dosimetry of in vivo radiation exposure being verified for the first time in cancer patients undergoing radiotherapy [5]. Using kinetochore antibodies, we also demonstrated that the great majority of MN induced by ionising radiation were kinetochore negative whilst those induced by spindle poisons were kinetochore positive (as one would expect given that radiation causes chromosome breakage leading to acentric chromosome fragments). This coincided with the beginnings of the era of molecular cytogenetics when the specific nature of chromosome lesions could be analysed using molecular (antibody or DNA) probes. At this time I recall giving a seminar at Flinders Medical Centre titled “The Micronucleus Assay at its Limits” as I had honestly thought that we had completely optimised the system and subconsciously I was ready for a new challenge.

I had made up my mind to do some further postdoctoral training to learn molecular biology techniques in the emerging fields relating to cloning of DNA repair genes and molecular

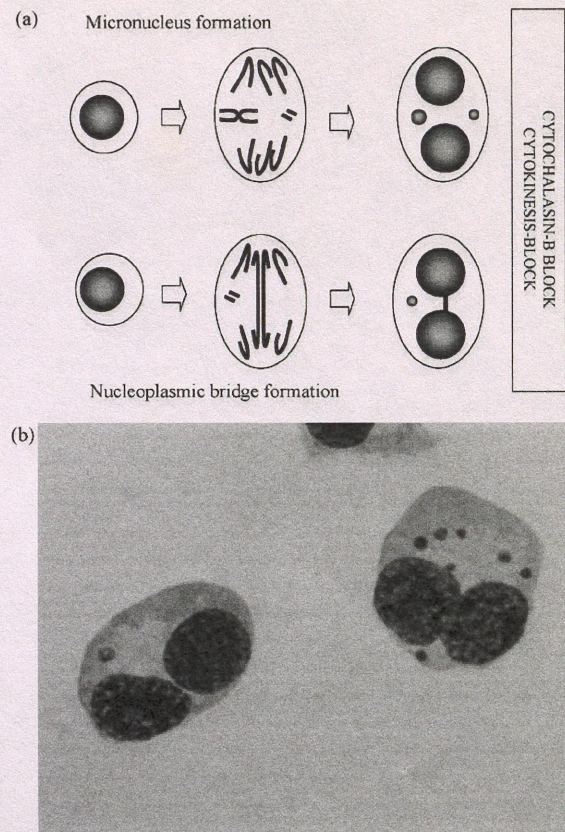


Fig. 1. The CBMN assay. (a) Micronucleus (MN) and nucleoplasmic bridge (NPB) formation in cells undergoing nuclear division. MN originate from either lagging whole chromosomes or acentric chromosome fragments. NPBs originate from dicentric chromosomes that may be caused by mis-repair of double strand DNA breaks or telomere end fusions. These events can only be observed in cells completing nuclear division which are recognised by their binucleated appearance after cytokinesis-blocking with cytochalasin-B. (b) Photomicrograph of binucleated cells with one micronucleus (left) and seven micronuclei (right).

analysis of gene mutations. Two of the leading laboratories in these fields at the time were Tony Carrano's group at the Lawrence Livermore National Laboratory and Bryn Bridges' and Alan Lehmann's group at the Medical Research Council Cell Mutation Unit (MRC CMU) in Sussex, UK. I visited both laboratories prior to making my decision. Tony Carrano thought it essential that I should continue with the MN assay and suggested that I work under Joe Gray's guidance to develop a flow cytometric method for the lymphocyte assay, whilst Alan Lehmann and Bryn Bridges were happy to take the risk of letting me loose in the molecular biology field which effectively clinched the deal. In hindsight I would have been happy to do both projects if it had been at all possible.

My experience at the MRC CMU proved to be a very positive and unforgettable one. The project involved cloning the *rad4* gene by complementation. I used the mutant *rad4.116* of the fission yeast *Schizosaccharomyces pombe*, which is temperature-sensitive for growth and sensitive to the killing actions of both ultraviolet light and ionising radiation. With the help of Tony Carr's expertise in transfection of fission yeast I isolated a couple of clones that were resistant to elevated temperature, and these were eventually used to clone the *rad4* gene in *S. pombe* [6]. The postdoc year in Sussex gave me important insights into the use of molecular biology in mutagenesis research and a feel for the international nature of research. It also allowed me to meet for the first time most of my “heroes” in the field of chromosomal mutagenesis including

Professor A.T. Natarajan, who had written many seminal papers in the field of radiation-induced chromosome aberrations and had examined my Ph.D. thesis, Professor Hans Stich who had pioneered the use of the buccal micronucleus assay, and Professor John Heddle who had first described the conventional MN assay in lymphocytes amongst others.

6. Blossoming of the micronucleus assay

A special event during my year in the UK was the "The Micronucleus Workshop" that David Scott and John Ashby had organised in Macclesfield (UK) to bring together the key experts in the MN field. Later in the year I participated in the Automated Micronucleus Workshop in Milpitas, California, in which the first successful automation prototypes of the erythrocyte MN and the lymphocyte CBMN assay were described and exhibited. An invitation the following year to present my work on the CBMN assay at the International Conference on Mutagenesis (ICEM) in Cleveland (USA) was unforgettable, as I was stunned and pleasantly surprised to find such an overwhelming interest in this presentation. I had not yet realised the significance and impact of this work, and I would not have guessed the extent to which the CBMN assay would be adopted worldwide. From thereon my working life was transformed, dictated as it was by the need to further develop and validate the use of this assay.

After this euphoric postdoctoral experience overseas I took up a position as a radiation biologist at the Australian Nuclear Science and Technology Organisation in Sydney, which unfortunately at the time was being restructured to become more commercially focussed. Regrettably, the importance of scientists relative to business managers had been declining for some years, and the focus on science was being steadily diluted. At about the time I began looking for positions elsewhere it happened that Dr. Ivor Dreosti at the Human Nutrition Division of the Commonwealth Scientific and Industrial Research Organisation of Australia (CSIRO) was seeking a postdoc to develop a new research area aimed at investigating the mitigating effects of dietary factors on oxidative stress, as well as at determining the genotoxic potential of different diets. I reasoned that it would be interesting to understand how diet might affect base-line DNA damage rates, and also to explore whether it had any radiation-protective effects. During my initial year at CSIRO, I simply established the lymphocyte CBMN assay there and completed the first improvement by using it in combination with cytosine arabinoside in G₁ to convert excision-repairable lesions directly into MN within one cell cycle [7]. I also established the bone-marrow erythrocyte MN assay at CSIRO and demonstrated the DNA-damaging effect of meat cooked at high temperatures. Collaboration with Youichi Odagiri revealed the possibility of scoring *in vivo*-induced DNA damage in normoblasts, myeloblasts and lymphoblasts in mouse bone-marrow cells using the CBMN assay in short-term *ex vivo* culture of bone-marrow cells following the addition of cytochalasin-B [8]. An interesting aspect of this study was the elevated radiation sensitivity of myeloblasts, which is consistent with observations on the reduction in neutrophil cell counts following radiation exposure. A notable and enduring experience during this period was the 1992 inaugural International Conference in Environmental Mutagenesis in Human Populations in Cairo, which was admirably organised by Wagida Anwar and her colleagues. I had the privilege of sharing in Frits Sobels' 70th birthday celebrations on the Nile. For me, having the opportunity to meet the founding editor of *Mutation Research* for the first time and to share this special occasion with him and close friends was a truly unforgettable experience.

7. Micronucleus assays in the study of nutrition

The line of research I chose to pursue at CSIRO Human Nutrition was inspired by the seminal work of Bruce Ames and Jim MacGregor showing that erythrocyte MN frequency in humans is strongly affected by folate status [9–11]. Between 1992 and 2000 we extended these observations by showing that the MN frequency index in lymphocytes was also associated with plasma vitamin B12, folate and homocysteine status in healthy adults, and that the MN frequency index in lymphocytes could be reduced by supplementation with folic acid and vitamin B12 [12–14]. The positive association with homocysteine was particularly important because it showed that DNA damage was associated with a known risk factor for cardiovascular disease (CVD) supporting the DNA damage hypothesis of CVD [15]. Evidence for this association was later strengthened by the association of MN frequency in lymphocytes with CVD mortality [16].

These studies as well as *in vitro* studies showed that the concentrations of folate and vitamin B12 then considered to be adequate for the prevention of anaemia were lower than those associated with the minimisation of DNA damage (e.g. 150 pmol/l vs. 300 pmol/l, respectively, in the case of B12). This raised the concern that dietary requirements for the prevention of DNA damage are likely to be different from those for the prevention of deficiency diseases. The *in vitro* studies also demonstrated that small differences within the normal physiological range of folate concentration (e.g. 10 nmol/l vs. 60 nmol/l folic acid) caused as much DNA damage as 10–20 cGy of X-rays, a dose range which is 5–10 times higher than the annual allowed limit of radiation exposure. This meant that small differences in nutrient concentration can cause as much DNA damage as doses of a mutagen and carcinogen about which there are serious concerns. At this time I published a series of papers promoting the concept that recommended dietary intakes should factor in the prevention of DNA damage, given that it was becoming increasingly evident that damage to the genome is implicated in multiple disorders (infertility, developmental defects, immune dysfunction, cancer, accelerated ageing) and can be considered among the most fundamental causes of disease [17–19].

Growing recognition of the fact that deficiency in dietary factors can modify the genome is indeed one of the main reasons for the importance and emergence of the new field of nutrigenomics. Several papers have now been published on the impact of diet on DNA damage (e.g. [20,21,30]). However, an even more interesting twist to this science stems from the potential of differences in susceptibility to DNA damage to depend on inherited polymorphisms in genes involved in the folate/methionine metabolism pathways—i.e., from nutrigenetics. This was the dawn of a new phase in research, but exploration of the interactive impact of micronutrient status and genetic background on DNA damage and cellular viability would require new research tools.

8. The HUMN project

The year 1997 proved to be one of the most important in my scientific career because it was then that Stefano Bonassi and I, after a year of email correspondence, decided to launch the HUMN project at the ICEM in Toulouse. We were overwhelmed by interest internationally in the HUMN project which had as its primary aims the collation of data worldwide to determine the main variables affecting lymphocyte micronucleus frequency, the establishment of scoring criteria for this assay, the performance of an inter- and intra-laboratory slide scoring exercise, and a prospective study to test the hypothesis that MN frequency in lymphocytes predicts cancer risk. All these objectives were met successfully within the following 10 years, and the resulting publications are amongst the most cited in the MN field [22–28]. The prospective study did in

fact show that a mid- or high-tertile level of MN frequency predicted an increased cancer risk [24].

9. Cytome approach to micronucleus assays

From 2000 onwards our laboratory embarked on research to create and validate a new way to perform the cytokinesis-block

MN assay—the “cytome” approach. Using this method, not only micronuclei but also other DNA damage biomarkers that we have observed in binucleated cells (i.e., nucleoplasmic bridges and nuclear buds) are scored. Cells undergoing cell death by necrosis and apoptosis are also identified and counted. Fig. 2 shows the endpoints considered in the cytome approach.

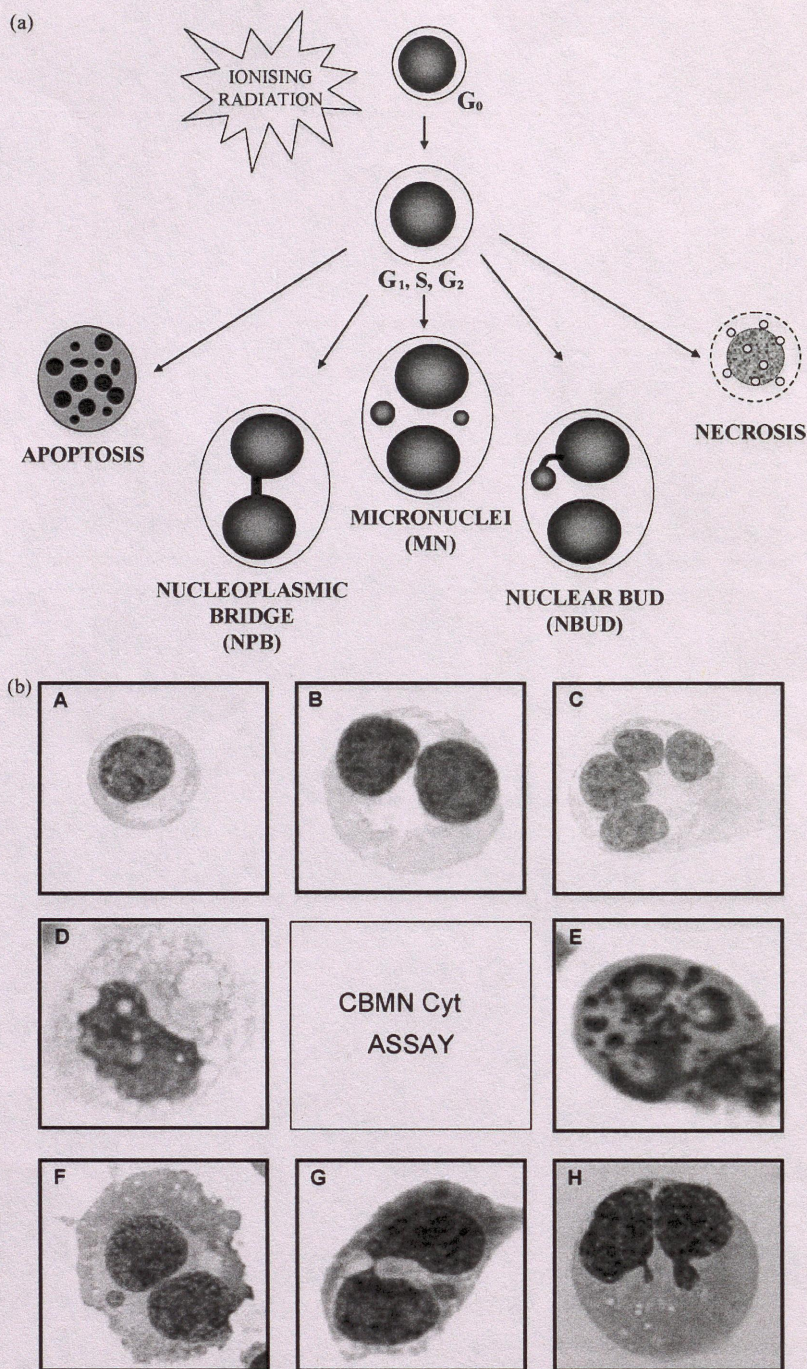


Fig. 2. The “Cytome” version of the CBMN assay. (a) The various possible fates of cultured cytokinesis-blocked cells following exposure to cytotoxic/genotoxic agents. Using these biomarkers within the CBMN assay it is possible to measure the frequency of chromosome breakage (MN), chromosome loss (MN), chromosome rearrangement, e.g. dicentric chromosomes (NPB), gene amplification (NBUDs), necrosis and apoptosis. In addition, cytostatic effects are readily estimated from the ratio of mono, bi and multinucleated cells. (b) Photomicrographs of the cells scored in the CBMN “cytome” (CBMN Cyt) assay. (A) Mononucleated cell; (B) binucleated cell; (C) multinucleated cell; (D) early necrotic cell; (E) late apoptotic cell; (F) binucleated cell containing one or more micronuclei; (G) binucleated containing a nucleoplasmic bridge (and a micronucleus); (H) binucleated cell containing nuclear buds. The ratios of mononucleated, binucleated, multinucleated, necrotic and apoptotic cells are used to determine mitotic division rate or nuclear division index (a measure of cytostasis) and cell death (cytotoxicity). The frequency of binucleated cells with micronuclei, nucleoplasmic bridges or nuclear buds provides a measure of genome damage and/or chromosomal instability.

The cytome approach was validated by the studies of Keizo Umegaki, Philip Thomas, Jimmy Crott, Shauna Brown, Will Greenrod, Bianca Benassi, Sasja Beetstra, and other scientists and students in my laboratory using models of oxidative stress and/or folate deficiency (references detailed in [29]). The observed positive correlation between micronuclei, nucleoplasmic bridges and nuclear buds indicates that these biomarkers of genomic instability are mechanistically related to one another. They could be explained by the breakage–fusion–bridge cycle model when the nutritional or exposure conditions generated double strand breaks in DNA and led to the formation of dicentric chromosomes [25,26,31–33].

The work of Kimura et al. [34] demonstrated the power of using the CBMN Cytome (CBMN Cyt) assay in investigating nutrient–nutrient and gene–nutrient interactions affecting the impact of folate and riboflavin concentration in individuals who are homozygotes for the C or T alleles of the C677T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene. The results from this work showed that increasing riboflavin concentration in a low folate background increased DNA damage (nuclear buds and nucleoplasmic bridges). Individuals homozygous for the T allele (which reduced enzyme activity) had reduced nuclear buds relative to the C allele homozygotes, indicating differential effects depending on nutrient concentration combinations and genotype. The observed sensitivity of the CBMN Cyt assay to nutritional and genetic factors confirmed its suitability as an important tool in the emerging exciting fields of nutrigenetics and nutrigenomics. The association of folate/methionine metabolism and DNA repair gene polymorphisms with the MN index in lymphocytes is becoming an active area of research in nutrigenomics and toxicogenomics [35].

The possibility of measuring nucleoplasmic bridges in the CBMN Cyt assay is of particular importance given that it is a potentially reliable assay for dicentric chromosome formation and therefore relevant to radiation biodosimetry [33]. It also allows a possible functional assay for telomere dysfunction when combined with telomere probes [29], given that telomere end fusions lead to dicentric chromosome formation and nucleoplasmic bridge formation. This has opened a possible new approach for studying the role of nutrition and other environmental factors on telomere dysfunction, which is virtually unexplored as yet.

10. The Genome Health Clinic concept emerges

In 2003 I put together various strands of thought that led to the Genome Health Clinic concept [30,36]. This concept is based on the premise that damage to the genome is fundamental to disease and can be diagnosed and nutritionally prevented. It was based on several lines of evidence showing that the CBMN cytome biomarkers are sensitive to nutritional deficiencies and excess, and that alterations to these biomarkers of DNA damage can be minimised by appropriate changes in diet and lifestyle and/or supplementation with micronutrients [12,14,36–38]. In 2005 we reported a cross-sectional study showing that at least nine micronutrients are associated with the micronucleus index [36], and studies are currently underway to test whether supplementation with a selection of these micronutrients can reduce the CBMN cytome biomarkers in a double-blind placebo-controlled trial. The Genome Health Clinic concept attracted much attention in Australia and led to the “DNA Doctor” story on the national science program “Catalyst” (<http://www.abc.net.au/catalyst/stories/s1381311.htm>). The opening of the first genome health clinic in Adelaide, South Australia, in June 2007 is a pioneering attempt to put this concept into practice (<http://www.csiro.au/partnerships/Reach100.html>).

10.1. The future

While this account is mainly about the past, I would also like to comment very briefly about the future. During the last 5 years we focussed more and more attention on the CBMN cytome approach as it became more evident that this reflected the genetic instability and regenerative potential of tissues within the body. For example, the work of El-Zein et al. suggested that nucleoplasmic bridges and nuclear buds might have a stronger association with lung cancer risk than do micronuclei in lymphocytes [39,40]. Philip Thomas's Ph.D. research in my laboratory showed that the MN cytome approach using buccal cells can also be remarkably effective in demonstrating the reduced regenerative potential of epithelial tissues in accelerated ageing syndromes, such as Down syndrome, and in Alzheimer's disease. These studies relied on biomarkers other than MN frequency (e.g. basal cell frequency) showing an association with these conditions [41–43]. In the HUMN project we have now launched an international collaboration on the buccal MN cytome assay to achieve the same objectives that were realised for the lymphocyte assay [44].

11. A personal afterword

It is important to note that none of the above would have been possible without the mentoring of Professor Alec Morley in my formative years and the support of friends, colleagues and family throughout the years. I hope that this account provides some inspiration and encouragement to the next generation of scientists in the fields of mutagenesis, radiation biology and nutritional genomics. In the past 2 years I have been fortunate to receive the Flinders University Convocation medal and the Environmental Mutagen Society Alexander Hollaender Award in recognition of these achievements which, in effect, reflect the success of efforts of many people whom I had the good fortune of knowing. Thus, this is also a story of valuable and rewarding scientific collaboration. I never met Alexander Hollaender but much has been written about this remarkable man and the spirit that he and such other pioneers of our field as Frits Sobels have engendered that has galvanised environmental mutagenesis research worldwide [45]. My story owes much to them also.

Conflict of interest

None.

Acknowledgements

None of the experiences and achievements would have been possible without the support of my wife Janet and my sons Nicholas and Benjamin who often have had to endure my absence so I could pursue my research interests with vigour. I would also like to acknowledge numerous staff members and colleagues who contributed to these experiences and achievements including Alexander Morley, David Turner, Kevin Trainor, Jack Dempsey, Alexander Kutlaca, Chris Matthews, Barbara Sanderson, Pam Sykes, Alexander Dobrovic, Monica McCarron, Sheila Phillips, Sonia Neville, Julie Turner, Felicia Bulman, Carolyn Salisbury, Philip Thomas, Maryam Hor, Varinderpal Dhillon, Will Greenrod, Jimmy Crott, Sasja Beetstra, Denise Furness, Bianca Benassi, Caroline Bull, Theodora Teo, Jing Wu and Nathan O'Callaghan. A special mention of thanks is due to my HUMN project coordinating group colleagues Stefano Bonassi, Errol Zeiger, Nina Holland, Wushou Chang, Claudia Bolognesi, Micheline Kirsch-Volders, Siegfried Knasmueller and all the scientists participating in the HUMN project activities. I am also grateful for the contributions of numerous other collaborators and the volunteers in our studies.

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References

- [1] P.I. Countryman, J.A. Heddle, The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes, *Mutat. Res.* 41 (December (2–3)) (1976) 321–332.
- [2] S.B. Carter, Effects of cytochalasins on mammalian cells, *Nature* 213 (January (5073)) (1967) 261–264.
- [3] M. Fenech, A.A. Morley, Measurement of micronuclei in lymphocytes, *Mutat. Res.* 147 (February–April (1–2)) (1985) 29–36.
- [4] M. Fenech, A.A. Morley, Cytokinesis-block micronucleus method in human lymphocytes: effect of in vivo ageing and low dose X-irradiation, *Mutat. Res.* 161 (July (2)) (1986) 193–198.
- [5] M. Fenech, J. Denham, W. Francis, A. Morley, Micronuclei in cytokinesis-blocked lymphocytes of cancer patients following fractionated partial-body radiotherapy, *Int. J. Radiat. Biol.* 57 (February (2)) (1990) 373–383.
- [6] M. Fenech, A.M. Carr, J. Murray, F.Z. Watts, A.R. Lehmann, Cloning and characterization of the rad4 gene of *Schizosaccharomyces pombe*; a gene showing short regions of sequence similarity to the human XRCC1 gene, *Nucleic Acids Res.* 19 (December (24)) (1991) 6737–6741.
- [7] M. Fenech, S. Neville, Conversion of excision-repairable DNA lesions to micronuclei within one cell cycle in human lymphocytes, *Environ. Mol. Mutagen.* 19 (1) (1992) 27–36.
- [8] Y. Odagiri, K. Takemoto, M. Fenech, Micronucleus induction in cytokinesis-blocked mouse bone marrow cells in vitro following in vivo exposure to X-irradiation and cyclophosphamide, *Environ. Mol. Mutagen.* 24 (1) (1994) 61–67.
- [9] J.T. MacGregor, R. Schlegel, C.M. Wehr, P. Alperin, B.N. Ames, Cytogenetic damage induced by folate deficiency in mice is enhanced by caffeine, *Proc. Natl. Acad. Sci. U.S.A.* 87 (December (24)) (1990) 9962–9965.
- [10] J.T. MacGregor, C.M. Wehr, R.A. Hiatt, B. Peters, J.D. Tucker, R.G. Langlois, R.A. Jacob, R.H. Jensen, J.W. Yager, M.K. Shigenaga, B. Frei, B.P. Eynon, B.N. Ames, 'Spontaneous' genetic damage in man: evaluation of interindividual variability, relationship among markers of damage, and influence of nutritional status, *Mutat. Res.* 377 (June (1)) (1997) 125–135.
- [11] B.C. Blount, M.M. Mack, C.M. Wehr, J.T. MacGregor, R.A. Hiatt, G. Wang, S.N. Wickramasinghe, R.B. Everson, B.N. Ames, Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage, *Proc. Natl. Acad. Sci. U.S.A.* 94 (April (7)) (1997) 3290–3295.
- [12] M. Fenech, J. Rinaldi, The relationship between micronuclei in human lymphocytes and plasma levels of vitamin C, vitamin E, vitamin B12 and folic acid, *Carcinogenesis* 15 (July (7)) (1994) 1405–1411.
- [13] M.F. Fenech, I.E. Dreosti, J.R. Rinaldi, Folate, vitamin B12, homocysteine status and chromosome damage rate in lymphocytes of older men, *Carcinogenesis* 18 (July (7)) (1997) 1329–1336.
- [14] M. Fenech, C. Aitken, J. Rinaldi, Folate, vitamin B12, homocysteine status and DNA damage in young Australian adults, *Carcinogenesis* 19 (July (7)) (1998) 1163–1171.
- [15] S. De Flora, A. Izzotti, Mutagenesis and cardiovascular diseases: Molecular mechanisms, risk factors, and protective factors, *Mutat. Res.* 621 (August (1–2)) (2007) 5–17.
- [16] E. Murgia, V. Maggini, R. Barale, A.M. Rossi, Micronuclei, genetic polymorphisms and cardiovascular disease mortality in a nested case-control study in Italy, *Mutat. Res.* 621 (August (1–2)) (2007) 113–118.
- [17] M. Fenech, Recommended dietary allowances (RDAs) for genomic stability, *Mutat. Res.* 480–481 (September) (2001) 51–54.
- [18] M. Fenech, Micronutrients and genomic stability: a new paradigm for recommended dietary allowances (RDAs), *Food Chem. Toxicol.* 40 (August (8)) (2002) 1113–1117.
- [19] M. Fenech, Genomic stability: a new paradigm for recommended dietary allowances (RDAs), *Forum Nutr.* 56 (2003) 97–100.
- [20] B.N. Ames, P. Wakimoto, Are vitamin and mineral deficiencies a major cancer risk? *Nat. Rev. Cancer* 2 (September (9)) (2002) 694–704.
- [21] B.N. Ames, Low micronutrient intake may accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage, *Proc. Natl. Acad. Sci. U.S.A.* 103 (November (47)) (2006) 17589–17594.
- [22] S. Bonassi, M. Fenech, C. Lando, Y.P. Lin, M. Ceppi, W.P. Chang, N. Holland, M. Kirsch-Volders, E. Zeiger, S. Ban, R. Barale, M.P. Bigatti, C. Bolognesi, C. Jia, M. Di Giorgio, L.R. Ferguson, A. Fucic, O.G. Lima, P. Hrelia, A.P. Krishnaja, T.K. Lee, L. Migliore, L. Mikhalevich, E. Mirkova, P. Mosesso, W.U. Müller, Y. Odagiri, M.R. Scarfi, E. Szabova, I. Vorobtsova, A. Vral, A. Zijno, Human MicroNucleus project: international database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes. I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei, *Environ. Mol. Mutagen.* 37 (1) (2001) 31–45.
- [23] S. Bonassi, M. Neri, C. Lando, M. Ceppi, Y.P. Lin, W.P. Chang, N. Holland, M. Kirsch-Volders, E. Zeiger, M. Fenech, HUMN collaborative group, Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human MicroNucleus project, *Mutat. Res.* 543 (March (2)) (2003) 155–166.
- [24] S. Bonassi, A. Znaor, M. Ceppi, C. Lando, W.P. Chang, N. Holland, M. Kirsch-Volders, E. Zeiger, S. Ban, R. Barale, M.P. Bigatti, C. Bolognesi, A. Cebulka-Wasilewska, E. Fabianova, A. Fucic, L. Hagmar, G. Joksic, A. Martelli, L. Migliore, E. Mirkova, M.R. Scarfi, A. Zijno, H. Norppa, M. Fenech, An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans, *Carcinogenesis* 28 (March (3)) (2007) 625–631.
- [25] M. Fenech, J. Crott, J. Turner, S. Brown, Necrosis, apoptosis, cytostasis and DNA damage in human lymphocytes measured simultaneously within the cytokinesis-block micronucleus assay: description of the method and results for hydrogen peroxide, *Mutagenesis* 14 (November (6)) (1999) 605–612.
- [26] M. Fenech, N. Holland, W.P. Chang, E. Zeiger, S. Bonassi, The Human MicroNucleus Project—an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans, *Mutat. Res.* 428 (July (1–2)) (1999) 271–283 (Review).
- [27] M. Fenech, W.P. Chang, M. Kirsch-Volders, N. Holland, S. Bonassi, Zeiger E, Human MicroNucleus project. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures, *Mutat. Res.* 534 (January (1–2)) (2003) 65–75.
- [28] M. Fenech, S. Bonassi, J. Turner, C. Lando, M. Ceppi, W.P. Chang, N. Holland, M. Kirsch-Volders, E. Zeiger, M.P. Bigatti, C. Bolognesi, J. Cao, G. De Luca, M. Di Giorgio, L.R. Ferguson, A. Fucic, O.G. Lima, V.V. Hadjidekova, P. Hrelia, A. Jaworska, G. Joksic, A.P. Krishnaja, T.K. Lee, A. Martelli, M.J. McKay, L. Migliore, E. Mirkova, W.U. Müller, Y. Odagiri, T. Orsiere, M.R. Scarfi, M.J. Silva, T. Sofuni, J. Surrallés, G. Trenta, I. Vorobtsova, A. Vral, Zijno A, Human MicroNucleus project. Intra- and inter-laboratory variation in the scoring of micronuclei and nucleoplasmic bridges in binucleated human lymphocytes. Results of an international slide-scoring exercise by the HUMN project, *Mutat. Res.* 534 (January (1–2)) (2003) 45–64.
- [29] M. Fenech, Cytokinesis-block micronucleus cytome assay, *Nat. Protoc.* 2 (5) (2007) 1084–1104.
- [30] M. Fenech, Genome health nutrigenomics and nutrigenetics—diagnosis and nutritional treatment of genome damage on an individual basis, *Food Chem. Toxicol.* 46 (April (4)) (2008) 1365–1370 (Epub 2007 July 5 Review).
- [31] K. Umegaki, M. Fenech, Cytokinesis-block micronucleus assay in WIL2-NS cells: a sensitive system to detect chromosomal damage induced by reactive oxygen species and activated human neutrophils, *Mutagenesis* 15 (May (3)) (2000) 261–269.
- [32] J.W. Crott, S.T. Mashiyama, B.N. Ames, M. Fenech, The effect of folic acid deficiency and MTHFR C677T polymorphism on chromosome damage in human lymphocytes in vitro, *Cancer Epidemiol. Biomark. Prev.* 10 (October (10)) (2001) 1089–1096.
- [33] P. Thomas, K. Umegaki, M. Fenech, Nucleoplasmic bridges are a sensitive measure of chromosome rearrangement in the cytokinesis-block micronucleus assay, *Mutagenesis* 18 (March (2)) (2003) 187–194.
- [34] M. Kimura, K. Umegaki, M. Higuchi, P. Thomas, M. Fenech, Methylenetetrahydrofolate reductase C677T polymorphism, folic acid and riboflavin are important determinants of genome stability in cultured human lymphocytes, *J. Nutr.* 134 (January (1)) (2004) 48–56.
- [35] G. Iarmarcovai, S. Bonassi, A. Botta, R.A. Baan, T. Orsiere, Genetic polymorphisms and micronucleus formation: a review of the literature, *Mutat. Res.* 658 (3) (2008) 215–233.
- [36] M. Fenech, The Genome Health Clinic and Genome Health Nutrigenomics concepts: diagnosis and nutritional treatment of genome and epigenome damage on an individual basis, *Mutagenesis* 20 (July (4)) (2005) 255–269.
- [37] H. Ishikawa, Y. Tian, T. Yamauchi, Influence of gender, age and lifestyle factors on micronuclei frequency in healthy Japanese populations, *J. Occup. Health* 45 (May (3)) (2003) 179–181.
- [38] L.J. Moran, M. Noakes, P.M. Clifton, R.J. Norman, M.F. Fenech, Genome instability is increased in lymphocytes of women with polycystic ovary syndrome and is correlated with insulin resistance, *Mutat. Res.* 639 (March (1–2)) (2008) 55–63.
- [39] R.A. El-Zein, M.B. Schabath, C.J. Etzel, M.S. Lopez, J.D. Franklin, M.R. Spitz, Cytokinesis-blocked micronucleus assay as a novel biomarker for lung cancer risk, *Cancer Res.* 66 (June (12)) (2006) 6449–6456.
- [40] R.A. El-Zein, M. Fenech, M.S. Lopez, M.R. Spitz, C.J. Etzel, Cytokinesis-blocked micronucleus cytome assay biomarkers identify lung cancer cases amongst smokers, *Cancer Epidemiol. Biomark. Prev.* 17 (May (5)) (2008) 1111–1119.
- [41] P. Thomas, J. Hecker, J. Faunt, M. Fenech, Buccal micronucleus cytome biomarkers may be associated with Alzheimer's disease, *Mutagenesis* 22 (November (6)) (2007) 371–379.
- [42] P. Thomas, N.J. O' Callaghan, M. Fenech, Telomere length in white blood cells, buccal cells and brain tissue and its variation with ageing and Alzheimer's disease, *Mech. Ageing Dev.* 129 (April (4)) (2008) 183–190.
- [43] P. Thomas, S. Harvey, T. Gruner, M. Fenech, The buccal cytome and micronucleus frequency is substantially altered in Down's syndrome and normal ageing compared to young healthy controls, *Mutat. Res.* 638 (February (1–2)) (2008) 37–47.
- [44] N. Holland, C. Bolognesi, M. Kirsch-Volders, S. Bonassi, E. Zeiger, S. Knasmüller, M. Fenech, The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps, *Mutat. Res.* 659 (July–August (1–2)) (2008) 93–108.
- [45] J.S. Wassom, Origins of genetic toxicology and the Environmental Mutagen Society, *Environ. Mol. Mutagen.* 14 (Suppl. 16) (1989) 1–6.