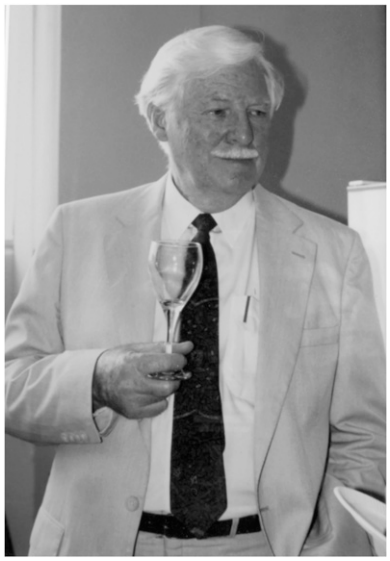
DOI: 10.1534/genetics.110.116624



Geoffrey W. Grigg
Photo provided courtesy of Fiona Grigg.

In Memoriam

Geoffrey W. Grigg (1926–2008)

GEOFFREY Grigg was recognized as a scientist with very wide interests and exceptional ability to exploit opportunities in both basic research and biotechnology. His long scientific career stretched from the early postwar dominance of microbial genetics to the sequencing of the human genome and beyond. *If there was a connecting thread or theme in his work it was undoubtedly DNA* but he also made a very significant discovery in cell biology before his first research in genetics. With Alan Hodge he studied the structure of the sperm tails of chicken spermatozoa. They discovered the 9 + 2 structure of the flagellum and published this in 1949, only a year after he obtained his first degree at Melbourne University in Australia (Grigg and Hodge 1949). This work went largely unrecognized and much later Irene Manton claimed priority in making the same discovery. In 1993, however, Pickett-Heaps and Martin put the record straight (Picket-Heaps and Martin l993; and see Grigg 1991).

In 1950 Grigg went from Melbourne to Cambridge as a Ph.D. student under the supervision of David Catcheside and initiated his study of mutation, which became a lifelong interest. Beadle and Tatum had revolutionized microbial genetics by isolating auxotrophs in the fungus *Neurospora crassa* and proposing the one gene–one enzyme concept. These auxotrophs frequently have a single nutritional requirement, so it is possible to easily measure back mutation to wild type by plating conidia (spores) on medium lacking the nutritional requirement. Many geneticists had started using this powerful new technique to measure mutation rates, for example, after mutagen treatment. It was always assumed that the background of nonmutant cells had no effect on the frequency of mutations. Grigg questioned this assumption and soon demonstrated that it was incorrect. The density of background cells can have a strong influence on the observed mutation frequency. This was published in *Nature* (Grigg 1952) and it soon became known as the "Grigg effect".

On his return to Australia, he continued his work with Neurospora for a while and then switched to *Escherichia coli* for further studies of mutation. He discovered that caffeine was a potent anti-mutagen in stationary phase cells, but not in growing cells. He explored the effects of caffeine on viability and DNA structure and discovered new methods for isolating mutant strains with deficiencies in DNA repair or with greatly altered spontaneous mutation rates (Grigg 1993). He studied in detail the effects of a large group of anti-cancer drugs, collectively known as bleomycins and phleomycins, on DNA breakage and cell death in *E. coli*. He then discovered that caffeine, and analogs of caffeine developed in conjunction with chemists in Canberra and Auckland, amplified these DNA damaging effects by a factor of 50-fold or more. This work with bacteria was followed up by examining the effects of the caffeine analogs on the anti-tumor activities of these drugs *in vivo*. Despite initial promising results with rats and mice, no funding was available to continue on to clinical studies. During this period, Grigg published at least 40 papers, participated in conferences worldwide, and became an internationally recognized authority in the fields of mutagenesis, DNA repair, and carcinogenesis. All this work was done in the Commonwealth Scientific and Industrial Research Organization (CSIRO) laboratories, North Ryde, Sydney, and in 1975 he became Chief of his Division.

In 1972, he accepted an invitation to work with Fred Sanger at the Medical Research Council (MRC) Laboratory of Molecular Biology (LMB) in Cambridge on

DNA sequencing. This experience with DNA chemistry proved invaluable to him when he became interested in DNA methylation. He was one of the first to recognize the significance of the proposal (by Holliday, Pugh and Riggs in 1975) that cytosine methylation in DNA might control gene expression and provide a new mechanism of inheritance. In the early experiments in this field, only a subset of methylation sites could be detected. In a later visit to the LMB in 1988, and after discussions with Dan Brown, Grigg realized that the presence or absence of methylation could be determined at all cytosine sites in a known sequence of DNA by the use of bisulphite. It had been previously shown that bisulphite converted cytosine to uracil, whereas 5-methyl cytosine was resistant. The treated DNA could then be cloned and the sequence of treated and untreated DNA compared to identify methylated sites. This bisulphite method was first used successfully in collaboration with Marianne Frommer's group at CSIRO in Sydney (Frommer *et al.* 1992). The method was soon being used worldwide, especially as the importance of DNA methylation in the control of gene expression was becoming more widely recognized. It has been said that the bisulphite method is one of the most important advances in DNA sequencing technology since Sanger invented the dideoxy DNA sequencing method. It is used in the massive Methylome sequencing project at the Sanger Centre near Cambridge, which will determine which cytosines are methylated in the whole human genome in different tissues.

Throughout his career, Grigg maintained a strong interest in the practical applications of basic scientific discoveries. His most remarkable attribute was to combine recognition of the implications of significant technical breakthroughs with the ability to persuade others to support their commercial development. In this way, he has made one of the most substantial contributions of any Australian scientist in promoting the biotechnology industry worldwide. He was among the first to recognize the importance of Kohler and Milstein's 1975 development of methods to generate monoclonal antibodies and established Australia's first antibody company, Bioclone, soon after. This company continues in operation today under Japanese ownership.

In 1986, he founded the company Peptide Technology, to exploit discoveries made in Denmark on efficient synthesis of peptides by the reversal of the protease reaction. A number of animal health products were commercialized using this approach, but the company soon turned its attention to other areas and in particular to using synthetic peptides to identify the site of interaction between the cytokine tumor necrosis factor (TNF) and neutralizing monoclonal antibodies. A patent was secured and the royalties from successful anti-TNF antibodies developed for treatment of inflammatory diseases proved of great financial benefit to the company.

In 1989, Grigg visited the LMB in Cambridge to catch up with progress on Gregory Winter's new approach for making human therapeutic antibodies. Grigg's visit was timely, as the work needed a major investment to advance the technology, and Grigg favored involving a start-up company. He persuaded Peptide Technology (later renamed Peptech) to provide seed funding, and Winter got the United Kingdom MRC to license the intellectual property. This formed the basis for the founding of Cambridge Antibody Technology Ltd. (CAT), with Grigg as Chairman of the Board. Soon thereafter, CAT started a program with BASF Pharma to develop an anti-TNF antibody, which became the first human therapeutic antibody (Humira) to be approved by the United States Food and Drug Administration. This antibody was marketed by Abbott for the treatment of rheumatoid arthritis and other inflammatory diseases and became a blockbuster drug (with sales of more than USD $6 billion in 2009), returning almost GBP £150 million in royalties to the MRC.

After CAT had been floated on the London Stock Exchange in 1997 with a valuation of GBP £100 million, Winter and LMB colleague Ian Tomlinson looked into developing antibody therapeutics on the basis of single (rather than paired) antibody variable domains. Again Grigg was an enthusiastic supporter and in 2001 persuaded Peptech to invest in a new start-up company. Domantis developed several lead products, including an anti-TNF single-domain antibody for Peptech, which entered clinical trials for psoriasis. In 2006, Domantis was bought by GlaxoSmithKline for GBP £230 million, with Peptech reaping many times its original investment. With ample cash and a product in clinical trials, Peptech itself became a target of acquisition (in 2009 by Cephalon for AUD $330 million).

Grigg had a strong interest in the bipeptide carnosine (beta alanyl-L histidine), which is widely distributed in animal tissues, but its function is largely unknown. In the CSIRO North Ryde laboratories it was shown that carnosine had the striking property of reversing the phenotype of senescent cultures of human fibroblasts near the end of their "Hayflick limit" to growth (McFarland and Holliday 1992). On the basis of a patent of which he is a co-inventor, Grigg founded yet another company, Beta Peptide Foundation Pty. Ltd. This company manufactured and marketed a range of skin care products containing carnosine (trade name BetaAlistine) that have been sold in Australia, Asia, and Europe.

In 2001, Grigg again turned his attention to DNA methylation by founding a new company called Human Genetic Signatures (HGS). One of its early aims was to greatly improve the bisulphite sequencing technique. The original method required the use of a significant amount of DNA, took 48 hr to perform, and degraded most of the DNA in the process. The new method requires one-thousandth of the DNA, degrades none of

it, and takes only a few hours to perform. HGS now sells bisulphite sequencing kits worldwide.

The never-ending advances in science bring with them an increasing complexity, documented in a huge literature. As a result most scientists become specialists in their chosen field of research. The polymath who crosses from one field to another is often undervalued precisely because those scientists in one field know little about the other. Geoffrey Grigg had a very unusual set of talents that allowed him either to undertake research or to collaborate with other scientists, in genetics, cell biology, molecular biology, developmental biology, peptides and proteins, immunology, and DNA technology. In reading the literature, he had an ability to spot what was novel and very significant for future exploitation in what had been discovered or what was being proposed. As important, he was charming and honest and his appearance matched his character. He looked the part of an Edwardian gentleman, well groomed and tailored with distinguished looks and a neatly trimmed moustache. It was easy to feel comfortable sharing scientific or business ideas with him. When used in combination, his talents proved formidable, especially in biotechnology, where his activities led to five biotechnology companies with combined valuations of more than USD $1 billion at acquisition and products ranging from research reagents to a blockbuster therapeutic antibody (and more in the pipeline). All those who knew him well appreciated his talents but many were probably unaware of the diversity of his interests and achievements.

Robin Holliday, Merilyn Sleigh, and
Gregory Winter

LITERATURE CITED

Frommer, M., L. E. McDonald, D. S. Millar, C. M. Colllis, F. Watt *et al.*, 1992 A genome sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. Proc. Natl, Acad. Sci. USA **89:** 1827–1831.

Grigg, G. W., 1991 The discovery of the 9 + 2 structure of flagella (cilia). BioEssays **13:** 363–370.

Grigg, G. W., 1952 Back mutation assay method in micro-organisms. Nature **169:** 98–100.

Grigg, G. W., 1993 A general method for selecting strains of Escherichia coli having altered spontaneous or induced mutabilities. Mutat. Res. **285:** 91–94.

Grigg, G. W., and P. J. Hodge, 1949 Electron microscope studies of spermatazoa 1. Morphology of the spermatazoan of the common domestic fowl. Aust. J. Sci. Res. **B2:** 271–286.

McFarland, G. A., and R. Holliday, 1992 Retardation of the senescence of cultured human diploid fibroblasts by carnosine. Exp. Cell Res. **212:** 167–175.

Pickett-Heaps, J. D., and A. Martin, 1993 An early, neglected description of the fibres (microtubules) in the eukaryotic flagellum. Protoplasma **176:** 14–16.