

Correspondence

Formic acid decalcification of bone marrow trephines degrades DNA: alternative use of EDTA allows the amplification and sequencing of relatively long PCR products

We recently reported a new method for the extraction of DNA from paraffin wax embedded bone marrow trephine biopsies.¹ The DNA extracted from EDTA decalcified bone marrow trephine biopsies using this method was sufficiently intact to allow the amplification and sequencing of relatively long polymerase chain reaction (PCR) products, including the 600 bp t(11;14) MTCA PCR product. A shorter 294 bp PCR product could only be amplified from six of 10 formic acid decalcified bone marrow trephine biopsies reported in a previous study by Provan *et al.*² These findings suggested a correlation between DNA degradation and formic acid decalcification, but required a comparative study for confirmation.

We have subsequently extracted DNA from 11 formic acid decalcified bone marrow trephine biopsies using our method and determined the quality of DNA using agarose gel electrophoresis and PCR analysis, as in our initial study.

The mean DNA yield from the formic acid decalcified blocks was twice that of the EDTA decalcified samples: 9.4 µg and 4.3 µg, respectively. This reflected the fact that the formic acid blocks contained approximately twice as much bone marrow trephine biopsy material as a result of the differences in practice between the two centres involved in the study (Exeter, EDTA decalcification; Southampton, formic acid decalcification). However, when the formic acid decalcified DNA samples were analysed by agarose gel electrophoresis, no high molecular weight DNA was detected; only a smear of degraded DNA was seen. In contrast, analysis of the EDTA decalcified bone marrow trephine biopsy DNA samples showed DNA ranging from 5 to 21 kb in length (fig 1).

It was not possible to amplify the 147 bp factor V PCR product from three of the formic acid decalcified DNA samples. The remaining eight samples generated very weak products compared with control DNA extracted from peripheral blood lymphocytes. This short PCR product was previously amplified successfully from all eight EDTA decalcified bone marrow trephine biopsy DNA samples.

The 482 bp BRCA 1 exon 11B product was only amplified successfully from one of the 11 formic acid decalcified samples. The intensity of the band seen on agarose gel electrophoresis was very weak compared with the control. Previously, all of our EDTA decalcified bone marrow trephine biopsy DNA samples were amplified successfully to generate this product.

Three formic acid decalcified samples yielded BRCA 1 exon 11A products (643 bp). However, the intensity of these products was so weak compared with the control that they were barely visible in the agarose gel. This relatively long PCR product had been amplified successfully using all EDTA decalcified bone marrow trephine biopsy DNA samples; five bands were of a similar intensity to the positive control, two were relatively weak, and one had to be diluted 1/20 to generate a band.¹

This comparative study strongly suggests that formic acid decalcification of bone marrow trephine biopsies causes DNA degradation, rendering specimens decalcified by this method unsuitable for use as a source of archival DNA.

Consequently, in view of the increased requirement for the use of molecular techniques in the diagnosis and monitoring of patients with lymphoma and leukaemia, the use of formic acid as a bone marrow trephine biopsy decalcifying agent should be reviewed. Decalcification with EDTA has been used routinely in the histopathology department at the Royal Devon and Exeter NHS Trust for several years and, despite the minor delay involved in tissue processing, causes no impairment of the quality of immunohistochemical and tinctorial staining in bone marrow trephine biopsies compared with formic acid decalcification. Directly as a result of the outcome of this comparative study, South-

ampton University Hospitals NHS Trust has now converted from the use of formic acid to EDTA for decalcification of BMT.

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1 Wickham CL, Boyce M, Joyner MV, *et al.* Amplification of PCR products in excess of 600 base pairs using DNA extracted from decalcified, paraffin wax embedded bone marrow trephine biopsies. *J Clin Pathol: Mol Pathol* 2000;53:19-23.

2 Provan AB, Hodges E, Smith AG, *et al.* Use of paraffin wax embedded bone marrow trephine biopsy specimens as a source of archival DNA. *J Clin Pathol* 1992;45:763-5.

Book reviews

Therapeutic Interventions in the Complement System. Lambris JD, Holers VM, eds. (£90.00.) Humana Press, 2000. ISBN 0 896 03587 5

The title misled me at first. At last, how to treat my complement deficient patients with more than antibiotics and vaccination. Nope, the back cover hyperbole quickly dashed that thought. What is presented here is a topical review of ways to inhibit complement and its inflammatory role in many diseases.

The essential role of complement activation is being defined in an increasing number of human diseases. This book details the complement system itself and the search for synthetic or natural inhibitors of the inflammatory pathways of complement. The first chapter gives a concise overview of the complement system and the role of physiological inhibitors. It also summarises several diseases in which turning off complement could be beneficial—for example, ischaemia/reperfusion injury, autoimmune renal disease, and transplantation. It is also the only place in the book where the possible side effects of such treatment are mentioned in any detail. Because the bulk of *in vivo* research has been performed in animal models, the effects on immunity to infection and immune complex mediated disease in humans needs very careful consideration in planning clinical trials, but this subject receives little attention.

Subsequent chapters are consistently structured, highly detailed accounts of different areas of the complement system. The chapters do not divide up into the usual "alternative", "classical", and "lectin" pathways (although the membrane attack pathway is dealt with in its entirety), mimicking the layout in text books, but rather group components with similar physiological roles. Each chapter goes on to explain the rationale behind developing inhibitors relevant to its own part of the pathway or group

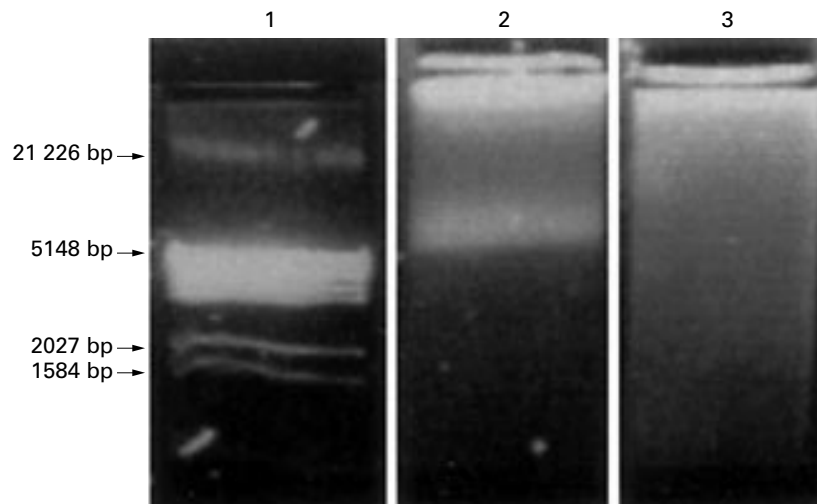


Figure 1 Agarose gel electrophoresis of DNA extracted from EDTA and formic acid decalcified bone marrow trephine biopsies. Lane 1, DNA size standard; lane 2, DNA extracted from an EDTA decalcified bone marrow trephine biopsy; lane 3, DNA extracted from a formic acid decalcified bone marrow trephine biopsy.

of components, how candidate agents are being developed, the use of the agents in animal models, and summaries of any clinical trials up to 1999. The only chapter that meanders a bit is that covering CR3 and CR4 because little bits of therapeutics are mixed in with the structure/function background.

The CR3/CR4 chapter is, however, deserving of a positive comment, because it is the only one that covers enhancing the anti-tumour role of complement. Considering that tumour expressed complement receptors play a major role in tumour escape from host defences, more could have been included. This subject, on the other hand, goes against the thrust of the rest of the book; so why do we get an excellent section on only one aspect of this field of work?

Although the basic activation pathways are described in each chapter, the emphasis varies and there is surprisingly little repetition of fine detail between chapters, showing that the editors have done their job well. Cross referencing within the book is limited and pertinent. The chapters that contain a few lines of summary or conclusions at the end get an extra gold star. I find this very useful in books of this calibre because some areas contain more detail than is needed by an individual reader (yes, I skipped bits!), and the summary lets you know if anything crucial has been overlooked.

The back cover seems to emphasise the coverage of "the new ELISA assays" for assaying complement products. The chapter entitled "Evaluation of complement inhibitors" details (you get a full methodology) the use of haemolytic assays for investigating the effect of putative inhibitors on individual components. It also covers the pros and cons of ELISAs, but anyone looking for details of ELISA methods will be disappointed.

In summary, there is excellent coverage of the structure and function of complement, broken down into slightly novel but rational areas. Details of how and why new therapeutic agents are being developed is comprehensive, as is the use of animal model studies. Clinical information is necessarily scant, and speculation about future developments fills up the gaps left by the book's title. I see no point in trying to decide specifically whom this book is aimed at because the contents will benefit basic scientists and a wide range of clinicians alike.

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The Molecular Basis of Cell Cycle and Growth Control. Stein GS, Baserga R, Giordano A, *et al.*, eds. (£45.50.) Wiley, 1998. ISBN 0 471 15706 6.

In recent years, there has been an explosion in the number of publications about the mechanisms that control the cell cycle and how their

deregulation can lead to cellular atypia and potentially carcinogenesis. It has become increasingly hard to find review articles that are both up to date and that look at the cell cycle in its entirety. This book, we are delighted to say, attains these criteria. While it starts at the beginning of the study of the cell cycle and attributes important findings to leading investigators, it takes the reader on a journey through the controlling mechanisms of the cell cycle, gradually increasing the detail and amount of information in this very complex subject. Each chapter is written in such a way that it stands alone, providing a rounded review of the topic in question, and yet the chapters also roll together building upon each other.

The first few chapters are devoted to the actions that take place in the different phases of the cell cycle and how these stages link to each other as the concentrations of the associated proteins rise and fall. PL Puri *et al* provide a comprehensive overview of the molecules involved in the cell cycle and how these interact to regulate its progression. Thankfully, they also differentiate between the nomenclature used for genes and proteins associated with the yeast cell cycle and those used for mammalian cells, an area that often causes confusion and unfortunately leads to the erroneous interchange of the two sets of molecules. G Prem Veer Reddy and later Greenfield Sluder *et al* expand upon the mechanism of action and regulation of DNA synthesis and mitosis, respectively; areas that are often glossed over in cell cycle reviews. Gary Stein *et al* elaborate on the transcriptional control of gene expression as the cell traverses from one phase to another and, in particular, they describe how this is used to ensure cell fidelity at the multiple checkpoints through the cycle. This is followed by a lengthy article by David Denhardt, who discusses the reasons why a cell either does or does not proliferate, the effect of exogenous and endogenous stimuli, and the cascade of events that occurs from the initial stimulus to the cell dividing.

The latter part of the book changes its emphasis slightly and looks at the ultimate outcome for a cell: differentiation or death. M Cristina Cardoso and Heinrich Leonhardt highlight the information currently available about the often forgotten act of terminal differentiation, something which should not of course be confused with cell quiescence. They continue to discuss the mechanisms involved in the decision of a cell to apoptose and provide evidence of the dual role that some molecules play in proliferation, differentiation, and apoptosis. Their final contribution is to provide an excellent review of DNA methylation; the current understanding and its role in carcinogenesis.

Another topic that has often led to confusion is cell senescence: how this differs from terminal differentiation and its relation to apoptosis. These concepts are clarified by Judith Campisi, who discusses the need for a finite cell life span and how some cells can bypass these protective mechanisms and become immortalised.

At first glance the final chapter by Bruno Calabretta and Tomasz Skorski does not appear to fit into the theme of the book. However, they use a chronic myeloid leukaemia model as an example of how genes and oncogenes associated with the transformation and maintenance of this disease can be targeted using antisense DNA. Hence, they show that the in depth study of the mechanisms controlling the cell cycle, and how these are altered in tumour cells, is not only of general interest but has great potential in the treatment of malignant disease.

The book makes very good use of diagrams to clarify the text; in particular, there are several colour plates in the middle of the book of both photomicrographs and diagrams, which are replicated in black and white at the relevant point in the text. As with any multi-author book, there is repetition of information, particularly because the cell cycle is introduced at the beginning of each chapter. This does not detract from the book, in fact it makes it easier when reading about one particular aspect of the cell cycle, and if needs be one can always skip over these introductions.

This is a well written and constructed book on factors that influence cell cycle and growth. It is extremely well referenced and we would recommend it to any one with an interest in the cell cycle.

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Books received

Cell Encapsulation Technology and Therapeutics. Kuhtrieber WM, Lanza RP, Chick WL. Birkhauser, 1999. ISBN 3 7643 4010 X.

Infectious Disease Pathology: Clinical Cases. Woods GL, Walker D, Winn W, *et al.* Butterworth Heinemann, 1999. ISBN 0 7506 9673 7.

Gene Quantification. Ferre F, ed. Birkhauser, 1998. ISBN 3 7643 3945 4.



The Molecular Basis of Cell Cycle and Growth Control. :

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